CELL MEDIATED IMMUNITY IN PROTEIN ENERGY MALNUTRITION

THESIS

FOR

M. D. (PEDIATRICS)

BUNDELKHAND UNIVERSITY
JHANSI

1990 **SURENDRA KUMAR**

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C E R T I F I C A T E

This is to certify that the present study entitled, "A CELL MEDIATED IMMUNITY IN PROTEIN ENERGY MALNUTRITION", has been carried out by SURENDRA KUMAR under my direct supervision and guidance. All the findings have been checked and verified by me from time to time.

He has put in the necessary stay in the department according to University regulations and also fulfilled all the conditions necessary for the submission of thesis.

Dated : 06.4.1990

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ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Dr. Ramesh Kumar, M.D.,D.C.H., Professor and Head of Department of Paediatrics, M.L.B. Medical College, Jhansi, for his unfailing help and advice extented to me at every stage of my humble venture. The entire work bears the stamp of his thinking.

I express my humble gratitude to Dr.(Mrs.) Sheela Longia, M.D., Reader, Department of Paediatrics, M.L.B. Medical College, Jhansi.

I acknowledge the help and stimulation given to me by Dr. A. Kaushik, M.D., Lecturer, Department of Paediatrics, M.L.B. Medical College, Jhansi.

I also express my sincere and heartful thanks to Dr. R.L. Sethi, M.D.,D.C.H., Lecturer, Department of Paediatrics, M.L.B. Medical College, Jhansi.

I deeply appreciate all my colleagues and friends for their co-operation and criticism that I was constantly in need of.

I am highly indebted to all my family members for their forebearance and understanding during this gestation.

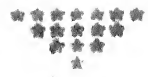
For the laborious and exacting task of transcribing illegible writing to fair type, I must thank Mr. K.M. Thomas.

Last, but certainly not the least, this study would not have been possible without the active participation of my wife Dr. Sheilly, I shall remain highly indebted for ever.

Dated :

(SURENDRA KUMAR)

C O N T E N T S



INTRODUCTION

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Protein Energy Malnutrition (PEM) poses a great challange today to most developing countries. It is estimated to be present in over 100 million children under the age of 5 years in economically derived areas of Africa, Asia and South America. The incidence of florid form of malnutrition such as marasmus and kwashiorkor has been estimated by Jayalaxmi and Gopalan (1958) to be 1 - 2% of the total child population.

The relation of diet and host resistance was mentioned in the ancient puranic scriptures in India. Nutrition is an important part of mosaic that determines the biological gradient and natural course of disease including infection. In the past 50 years, the observation that thymus is involuted in people dying of starvation activated the study of nutritional immunology. Necessary impetus to the topic was given by Chandra (1972) in India and by Smyth et al (1971) in Africa.

Health workers in under-privileged communities have noted the mutually aggravating interaction of malnutrition and infection. This is particularly evident in young children in whom nutritional status is a critical

determinant of both mortality and morbidity. In the rural area young children, 1-24 months old, whose weight is less than 60% of the standard carry a mortality risk of 18% in contrast to less than 1% among the well nourished. Infection acquires considerable importance in the situation where poverty and unhygienic environment prevail.

Malnutrition produces morphological and functional changes in virtually all organs. The severity of nutrition-related dysfunction depends in part on the rate of cellular proliferation and renewal and on protein synthesis in the tissues. In this regard, the lymphoid system is most vulnerable. It is not surprising then that undernutrition has profound adverse effect on mucosal and systemic immunity. Profound thymic atrophy was identified as one of the striking histopathological feature of severe malnutrition more than 50 years ago. In malnutrition thymus is small with ill-defined demarcation between the cortex and the medulla. There are fewer than normal lymphoid cells. In the intestine this includes flattening of villi. Smythe (1971) and Chandra (1972) have documented small lymphoid tissue in gut wall in malnutrition.

Host defenses include such diverse system as non-specific immunity (Saliva flow, bactericidal enzyme), humoral immunity (antibody, complement mediated lysis), cellular immunity (such as phagocytosis, T-lymphocyte

killer cells) and mucosal immunity (antibodies in secretion). These immune system vary in their response to nutritional stress, yet their combined function provides overall host defenses. On a world wide basis protein calorie malnutrition is the most common cause of acquired immune deficiency.

Humoral immune system is comprised of soluble serum protein, in particular immunoglobulins (B-cells, IgG, IgM, IgA) and the complement system. The B-lymphocyte is composed of finger like cytoplasmic process. B-lymphocyte posses a variety of independent receptors having more than one class of immunoglobulins on their surfaces. The nature of B-lymphocyte is incompletely understood, but it appears to be composed of a protein or glycoprotein that is resistant to trypsin and is distinct from the immunoglobulin molecules synthesized by the cell. Receptor on the cell surface make EAC rosettes with sheep red blood cells. B-lymphocyte have their origins in extrathymic sites (e.g. bone marrow and fetal liver) and are presumably not processed by the thymus. B-lymphocytes mature to become antibody producing plasma cells.

Cellular immunity is produced by sub-population of lymphocytes which bind to foreign protein components of pathogens. This binding process can result in direct killing of pathogen or more importantly, attraction and activation of macrophage into killer cells which then

engulf and destroy pathogens. In malnutrition, both number and functional capability of T-lymphocytes is markedly reduced, as is the delayed hypersensitivity response to intradermal and contact allergens.

T-cells are detected by their adherence to unsensitized sheep red blood cells. Immune response depends upon their cellular interaction. Since such interaction depends on surface properties, it is tempting to relate the mechanism of the immune response to cell surface receptors. Again, since presentation of antigen to appropriate lymphoid cells is the key to the immune response it is important to understand the relation between cell receptors and the binding of antigen. Once a specific immune response has been generated against an antigenic agent, the phagocytic cells assume an important role in antigen disposal in both humoral and cellular immunity.

As a test of cellular immunity, contact sensitization to 1 chloro, 2, 4 dinitrochlorobenzene (DNCB) offers several advantages over intradermal tests. Reliance upon previous exposure to allergen is unnecessary and circulating antibodies do not develop with contact sensitization, which renders it a more exact test of cellular immunity.

Aim of the present study was to evaluate the cell mediated immune response and humoral response in children having protein calorie malnutrition. This evaluation was done by :-

1. Assessing T-lymphocyte count and comparing it with the T-lymphocyte count in healthy subjects.

2. Evaluating the correlation between malnutrition and B-cell count.

3. Evaluating the correlation between T-lymphocyte count and 2, 4-dinitrochlorobenzene skin test.

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REVIEW OF LITERATURE

REVIEW OF LITERATURE

Protein Energy Malnutrition

Infantile malnutrition due to protein and calorie deficiency must have been common in large parts of the world for centuries, but attention seems to have been focussed upon it only in the early years of this century. Histologically Marasmus (Greek Marasmos, Wasting) was recognised for hundreds of years as being associated with gastroenteritis, a major contributor to high infant mortality. The term 'Kwashiorkor' (taken from Ga language of Ghana) was given by Cicely William (1963) and she recognised that this was a disease characterized by skin and hair changes, oedema, moon face, fatty liver, hypo-albuminaemia and psychomotor changes. Waterlow (1948) and Jelliffe et al (1954) used the term 'Nuger baby' to describe, obviously a similar condition as kwashiorkor found in West Indies, where dermatosis was uncommon though oedema was prominent.

Jelliffe (1959) coined the term 'protein calorie malnutrition (PCM) of early childhood' to include the mild and moderate degrees and all the clinical types of the severe degree of malnutrition.

There was a short lived effort through W.H.O. to introduce the term 'Protein Calorie deficiency diseases' but this was abandoned by the expert group meeting in 1970 in favour of Protein Calorie Malnutrition. To replace the term 'calorie' by 'Joule' as a unit of energy lead to the general use of word 'Protein Energy Malnutrition (PEM)! To emphasize that this was part of the overall energy crisis of mankind, the term energy protein malnutrition or EPM was used by some to give the needed stress to energy deficit (McLaren, 1973).

Good nutrition is the basic component of health. Protein energy malnutrition is the most wide-spread nutritional disorder amongst pre-school children. It is one of the great offenders of childhood morbidity and mortality in the tropical world. PCM has been a major nutritional problem of most countries, mortality being 20 - 30 times higher in developing countries. In India itself 80 million children are malnourished and out of these, 3 - 4 million are suffering from severe protein energy malnutrition (Shah, 1976).

Ghai (1977) reported that 40% deaths in children could be attributed to malnutrition, even though same was often not listed as the primary cause of death in most of the studies.

Rao (1978) reported 1 - 2% incidence of marasmus and kwashiorkor in pre-school children. As many as 60-70% of the children suffered from mild to moderate degrees of PCM, as observed by the author.

Ghosh (1981) estimated that there were about 100 million pre-school children in India. Out of these 3 - 4 million suffered from severe degrees of malnutrition and probably 1 million of them die every year.

Classification

Various classifications of PEM have been given from time to time by various workers. Methods of assessing PCM in the community are : clinical, anthropometric and biochemical.

Gomez and his associates (1956) must be credited for giving the first classification of malnutrition using the actual body weight, expressed as a percentage of standard (expected) weight (Harvard, 50th percentile) for that age, as the basis of nutritional status.

Grade of malnutrition	Body weight
Normal	$\geq$ 90% of expected weight for age.
Grade I	89 - 75% " " "
Grade II	74 - 60% " " "
Grade III	$<$ 60% " " "

Its main drawback was that it assumed all children of certain age to have the same weight, irrespective of their size as measured by height.

Jelliffe (1966) modified Gomez's classification by including all cases with nutritional oedema (irrespective of the weight) in severe degree of malnutrition.

McLaren (1967) introduced a simple scoring system for classifying the severe forms of malnutrition based on all the three methods of assessment viz. clinical, anthropometric and biochemical.

McLaren classification

Sign		Points
Oedema		3
Dermatosis		2
Oedema + dermatotis		6
Hair changes		1
Hepatomegaly		1
S. Albumin(gm/100 ml)	Total serum protein(gm/100 ml)	
< 1.00	(< 3.25)	7
1.00 - 1.49	(3.25 - 3.99)	6
1.50 - 1.99	(4.00 - 4.74)	5
2.00 - 2.49	(4.75 - 5.49)	4
2.50 - 2.99	(5.50 - 6.24)	3
3.00 - 3.49	(6.25 - 6.99)	2
3.50 - 3.99	(7.00 - 7.74)	1
4.00	(7.75)	0

Score = sum of points.

A score of 0 - 3 is Marasmus;

4 - 8 is Marasmic kwashiorkor, and

9 - 15 is Kwashiorkor.

Either serum albumin or total serum protein is used for the sake of deriving a composite score.

Certain other classifications have been designed which use measurements that are taken by a simple apparatus. These classifications are based on the concept of age independent criteria. Such methods are supposed to be used in the field by unskilled personnel. Among them, Quacstick (Arnold, 1969) method makes use of height and mid arm circumference measurements.

The ratio of mid-arm circumference to head circumference measurement has been shown to be age independent, at least from 3 - 48 months, and remains unchanged with the variation in sex (Kanawati and McLaren, 1970).

Ratio of mid-arm circumference and head circumference	Status of nutrition
$\geq$ 0.310	Nutritionally healthy
0.310 - 0.280	Mild PCM
0.279 - 0.250	Moderate PCM
$<$ 0.250	Severe PCM

However, this method is thought to be less accurate and useful only for screening purpose.

The classification that appeared in the 8th report of FAO/WHO Expert Committee (1971) was originally prepared by the Wellcome Trust and is referred to as Wellcome Classification.

Wellcome Classification

Category	Oedema	Deficit in weight for height	Body weight as % of standard
Under weight	0	Minimal	80 - 60%
Nutritionally dwarf	0	Minimal	< 60
Marasmus	0	++	< 60
Kwashiorkor	+	++	80 - 60
Marasmic-kwashiorkor	+	++	60

Standard weight is taken as 50th percentile of the Harvard weight for age.

$$\text{Weight for height} = \frac{\text{Weight of patient}}{\text{Weight for normal subject with the same height}} \times 100$$

Wellcome Classification was probably the first in which an attempt was made to use weight for height, as well as weight for age ratios. Thus, the classification

delineated a specific entity viz., Nutritional Dwarfs. However, it had some notable deficiencies. It confused between the type and severity of malnutrition and as a result, kwashiorkor appeared to be less severe than the other two types of malnutrition. Again, gradation of deficit in weight for height was not quantitated.

Nutrition sub-committee of the Indian Academy of Paediatrics (1972) classified PCM into 4 grades using 50th percentile of Harvard Standard as a reference point. Classification of the sub-committee of Indian Academy of Paediatrics (IAP) is usually followed for research purposes. Nutrition sub-committee of Indian Academy of Paediatrics met in 1972 and recommended that the classification of malnutrition should be simple and based on weight loss and severity of symptoms. Since growth norms provided by The Indian Council of Medical Research (ICMR) did not reflect growth pattern of children from various socio-economic groups, 50th percentile of Harvard Standard, which was similar to the 50th percentile of the Indian children from upper socio-economic group, was used as reference standard in grading the nutritional status, in IAP classification.

Classification of I.A.P.

Grade	Weight expressed as % of reference standard
I	71 - 80%
II	61 - 70%
III	51 - 60%
IV	< 50%

Grades I and II were categorised as under-weight while grades III and IV corresponded to clinical status of marasmus. When nutritional oedema was present, letter K was suffixed to the grade denoting kwashiorkor. I-K and II-K would mean kwashiorkor and grade III-K and IV-K would correspond to marasmic kwashiorkor.

Waterlow and Ruti Shauser (1974) published a classification based on weight and height, thus accounting for past as well as present malnutrition. Waterlow maintained that weight for height was independent of age.

Grade	Stunting (Height for age)	Wasting (Weight for height)
0	> 95%	> 90%
1	95 - 90%	90 - 80%
2	89 - 85%	80 - 70%
3	< 85%	< 70%

Definition of PEM

W.H.O. (1973) gave the following definition of PEM : "A range of pathological conditions arising from coincident lack, in varying proportion of Protein and calories, occurring most frequently in infants and young children and commonly associated with infection". The condition concerned could be said to range in severity from mild to moderate degrees of malnutrition. It could, as well, be subclinical and only detected by anthropometric and biochemical tests.

Role of nutrition in immunity :

The relation of diet and host resistance has been mentioned in the ancient puranic scriptures of India. The concept that dietary factor influences health is not new. However, in recent years, it has become clear that nutrition is an important part that determines the biological gradient, the natural course of disease including infection.

Nutrition, immunity and infection are known to be closely linked. Inadequate nutrition can alter the immunocompetence and thus increase susceptibility to infection. Infection in turn can adversely alter nutritional status.

There are mainly two types of immune mechanisms which operate against infection. These are 'humoral' and 'cellular' immune mechanisms. Also, there are other non-specific defence factors such as lysozyme complement and opsonins which play an important role in determining resistance to infection. Phagocytic activity and bactericidal properties of leucocytes constitute the first line of defence. Alteration in one or more of these mechanism may be expected to increase susceptibility to infection.

IMMUNOLOGICAL SYSTEM

Immunological system is the part of host defence. Its primary function is to protect against invasion by infectious agent. The major cost of this protection is allergy, auto-immunity and/or rejection of organ transplant. Following are the major limbs of immunological system : T & B lymphocytes, phagocyte and complement.

Cell mediated immunity in malnutrition :

Most consistent change in immuno-competence in PEM is in cell mediated immunity. Vint (1937) reported that malnourished children invariably had evidence of thymic atrophy. There was cellular depletion with involution of thymus dependent areas in the spleen and lymphnode. This morphological alteration was reflected

in reduced activity of serum thymic factor which could be responsible for the depression of immune response.

Trowell et al (1954) studied immunological status in kwashiorkor. Autopsy of children with kwashiorkor revealed shrunken thymus. These authors inferred that malnutrition affected the immune response.

Scrimshaw et al (1968) recognized that there was a synergistic interaction between infection and malnutrition.

Phillips et al (1968) observed that severe infection was common in PEM.

Srikantia (1969) observed that mantoux test was often negative in children suffering from kwashiorkor inspite of the clear evidence of active tubercular infection. This observation led the author to conclude that cell mediated immunity was impaired in severe malnutrition.

Ramalingaswami (1969) stated that PEM exerted profound effect on cellular growth and functions. He observed that any cell and organ in the body was involved although to a variable degree. Organ with higher rate of cell renewal such as gut, bone marrow and lymphoid tissue were affected most. There was depletion of lymphocytes and cellular element of immune system.

Smythe et al (1971) demonstrated profound depression of thymolymphatic system and cell mediated immunity in malnutrition.

Edelman et al (1973) observed depressed inflammatory response and cell mediated response in PEM.

Ramalingaswami (1973) observed that malnutrition and infection singly or in combination contributed significantly to morbidity of infants and children in developing countries.

Bhaskaran et al (1974) studied cell mediated immunity by measuring the number of T lymphocytes and incorporation of 3H thymedine into lymphocytes following stimulation with non-specific mitogen PHA (Phytohemagglutinin). Authors found that cell mediated immune response was significantly depressed in malnutrition. They also observed that this response was unaltered in mild grades of malnutrition.

Reddy (1976) also reported that cell mediated response remained unaltered in mild to moderate degree of PEM. Kumar et al (1978) observed depressed cell mediated immunity (CMI) in children with PEM.

Reddy et al (1978) saw that nutrition, immunity and infection were inter-related. They showed that deficient nutrition could alter the immuno-competence, thus increasing the susceptibility to infection.

Puri et al (1980) reported that various parameters of cellular immunity were significantly depressed in severe PEM.

Chandra (1983) observed in his study that there was delayed cutaneous sensitivity to a battery of recall antigens or other chemical sensitizing agents.

Humoral immunity in malnutrition :

Majjar (1969) reported normal levels of immunoglobulins in PEM.

Chandra (1972) has reported slightly increased levels of immunoglobulin in PEM.

Chandra (1975) in his study observed that there was a reduced secretory antibody response to live attenuated measles and polio virus in malnourished children.

Puri et al (1980) observed that humoral immunity was not altered in PEM except in the presence of infection, when there was some increase in IgG level.

Udall (1982) reported that antibody response to those antigen which required the help of T lymphocyte and/or macrophage was compromised in malnutrition.

Phagocytosis in malnutrition :

Chandra (1972) showed in his study that energy production for phagocytosis was impaired but there was no demonstrable defect in phagocytes.

Chandra & Chai (1976) showed in their study that there was a reduced neutrophil response to pseudomonas polysaccharide in cases of malnutrition.

T and B cell lymphocyte :

Harris et al (1945) showed that lymphocytes were involved in the immunological system. It is now recognised that lymphocytes form an indispensible component of body immune system. Although peripheral blood lymphocytes account for only 0.2% of total lymphoid tissue in human body (Osgoad, 1954), yet there is a free and extensive migration of lymphocytes via blood and lymph, to various lymphoid organs, connective tissue and bone marrow. This continuous intermingling of a variety of lymphocytes makes them a perfect vehicle for the transportation and dissemination of viruses, bacteria, other antigens and antibody information throughout the body (Yoffey, 1964).

Study of Clamen et al (1966), Devis et al (1967), Millar and Michel (1968) indicated that at least two populations of lymphocytes were involved in most of the

immune responses and that they differed in their anatomic distribution. Cells of one population were the precursors of plasma cells. It appears that precursor bone marrow cells (prothymocytes) migrate to thymus gland where they are processed become functionally competant and are transported into lymphocyte compartment (Moore and Owen, 1967; Owen and Ritter, 1969; Owen and Raff, 1970; Stutman and Good, 1971). They were present in bone marrow but not in thymus and they corresponded to bursa dependent system of chicken. Another population was dependent on and derived from thymus. These two populations of cells were named as T cell (thymus dependent) and B cell (Bursa equivalent) (Rettest et al, 1969).

Graves et al (1973) showed that T cells were concerned with cell mediated (CMI) and B cell with humoral immunity.

T lymphocytes play a major role in the immune response of facultative organisms, e.g. tissue/organ graft and certain infections with viruses. T cells accounts for as many as 70% of the peripheral blood lymphocytes while 20 - 25% are B cells (Lukes et al, 1974). B lymphocytes mature to become antibody producing plasma cells and play a role in humoral immunity response (Rowland, 1975). Lymphocytes circulate 4 to 6 times a day.

Cellular membrane is not simply an elaborately constructed container of cytoplasmic organ. Rather it is a complex, highly mobile structure that functions in recognizing certain extra-cellular materials and in transmitting information generated by their recognition to sub-cellular organells. Cell surface characteristics of a lymphocyte and monocyte determines the way in which foreign material will be recognized and delt with, through an immune response. Antigen specific receptors of T and B cells are essential to generate specific immune responnes and may be even required for the distribution of antigen throughout the host (David et al, 1975).

T lymphocyte :

It appears that precursor bone marrow cells (prothymocytes) migrate to the thymus gland, where they are processed, becomes functionally competent and are then exported into peripheral lymphoid compartment (Moore and Owen, 1967; Owen and Ritter, 1969; Owen and Roff, 1970); Stutman and Good, 1971). Moreover, profound changes in cell surface antigens marks the stage of T-cell antigeny (Raff, 1971). Thymus compartment contains early thymocytes (T_{10}^+ T_9^+), common thymocytes (T_{10}^+, T_1^+, T_3^+, T_4^+, T_5^+) and mature thymocytes.

T lymphocytes are grouped broadly into modulator cells, effector cells and cells producing lymphokines.

Modulator cells are further divided into two categories; those that initiate (helper cells or inducer cells) and those that tend to terminate (supressor cells) immune response (Reinherz and Schlossman, 1980).

Peripheral T cell compartment contains mature (stage III) lymphocytes which give rise to peripheral T-cell inducer (IND) and cytotoxic/supressor (C/S) sub-sets.

Helper inducer T cells facilitate antibody production by plasma cell (B cells) and modulate interactions between lymphocytes and accessory cells through the release of lymphokines. The possible mechanism for subsequent termination of antibody production is the activity of supressor cells.

There appears to be a sub-population of inducer T cell which is required to induce the functions of T supressor lymphocytes (Morimoto et al, 1981).

In addition to modulatory lymphocytes there are other T lymphocyte as well, viz. cytotoxic effector cells. Cytotoxic effector cells destroy the target cells or provide negative feed back to inhibit antibody response or down regulate inflammatory response (Paul, W.E., 1980). The other function of T lymphocytes is the secretion of lymphokines. These low molecular weight substances

secreted by activated T lymphocytes affect the function of other cells in the surrounding environment. T cells secrete interferon that stimulates other cells to develop anti-viral activity. Macrophage migration inhibition factor secreted by stimulated T cells causes activation and immobilization of macrophages at the site of inflammatory response (Rocklin et al, 1980). The human immune system therefore consists of discrete sub-sets of T cells that are critical for immune homeostasis. It is the balance between effector and regulatory sub-sets that governs the outcome of antigen triggering. The inducer sub-set, is central for the activation of other T cells; B cells and macrophages, as well as for hematopoietic differentiation. Loss of these sub-sets leads to a variety of immunologic disorders, characterized by auto-immunity or immuno-deficiency.

The concept of opposing activity of the modulator lymphocytes is part of the theory of regulation of immune response (Teurog et al, 1981).

Inter-leukin-2 is a lymphokine that promotes activation and division of other T lymphocytes (Gillis, 1983). There is a reduction of circulating thymus dependent T lymphocytes in PEM (Chandra et al, 1983).

B lymphocytes :

B lymphocytes synthesize and excrete specific antibodies and serve as receptors for antigen. Plasma cells represent the extreme form of B cell differentiation. Earlier studies suggested that B cells could be distinguished from T cells by recognition of finger link cytoplasmic processes which were more numerous on B cells. B cells show single antigenic specificity on their surfaces when exposed to the relevant antigen (processed by a macrophage). Under the influence of signals from antigen specific T lymphocyte, B lymphocytes differentiate into plasma cells, which secrete antibody of the same specificity as originally found in its progenator. Lymphocytes having surface IgM, IgG, IgA, IgE, IgD make up the greatest number of B cells in the peripheral blood of adult. Nearly 15 - 30% of peripheral lymphocytes are identified as B lymphocytes. B lymphocytes can be shown to have cell surface receptors that interact specially with certain components of complement. Sheep red blood cells prepared under appropriate condition (EAC) form rosette with human B cells. Lymphocyte membrane possesses temperature sensitive, mobile cell surface components.

Immunoglobulins which are widely dispersed over the cell surface form a "cap" when cells are exposed, at 37°C temperature, to labelled anti-serums (essential to produce cross linking of the surface immunoglobulins).

"Capping" may be effective in producing conformational changes of cell surface receptors permitting necessary redistribution of these important molecules. Such 'capping" may be a part of the process of differentiation of lymphocytes to plasma cells (Craves et al, 1973). Adherence of lymphocyte and macrophage to the indicator system is known as EAC rosette. B cells are commonly identified by immunoglobulin on sig marker.

Phagocytes :

Third component of immune system comprises of fixed phagocytes of reticulo-endothelial system as well as wandering phagocytes (polymorpho-nuclear or mononuclear myeloid cells). The most primitive activity of phagocyte is ingestion of foreign substance which may be degraded, killed or simply transported away from the threatened tissue. The phagocyte is able to secrete at least 50 products into its environment. Large mononuclear phagocytic cells also possess receptors for FC component of immunoglobulins and modified component of complement. The functional relation of these receptors on phagocytes is more readily recognized than those on B cells. Macrophages do not synthesize immunoglobulins.

Method of detection of macrophages by cytophilic nature of antibodies, measures the binding of unaggregated

immunoglobulins, free of antigen, to macrophages. An opsonic property of antibody can be detected by binding of antigen-antibody complex to macrophages. Macrophages possess receptors for complement C_3b, but it is less clear which other component is truely important. The macrophage has two critical roles - first is that it initiates specific immune response. Second function of macrophage is that its products exert a modulatory function on inflammatory response. Enzyme inhibitor such as plasmin and alpha 2 macroglobulin blocks the action of proteolytic enzymes. Macrophages product, prostaglandin, has been incriminated in suppression of lymphocyte function in vitro (Rice et al, 1979). The T lymphocyte must "see" the antigen presented at the surface of macrophage that has the same histocompatibility antigen (Unanu et al, 1980).

Interlukin-1, a macrophage secretory product acts in vitro as a stimulent for the proliferation of certain T lymphocytes and production of lymphokines (Bendtzenk, 1983).

Complement :

The complement pathways consist of a series of proteins in serum with the following features -

1. Sequential activation of inactive precursor (zymogens).

2. Activation of an increasing number of molecules in subsequent steps of the sequence (Cascade).

3. Amplification of propogation of inflammation by products of activation. There are two pathways of complement system - classic pathway and alternate pathway. The complement system is invariably affected in PEM. The total haemolytic complement activity may be reduced as also the level of C_3. Infection may produce acute phase reactants, increase in complement activity, but more often a further depression in the concentration of complement protein, partly as a result of consumption in the antigen - antibody reactions. Due to the change in complement system in PEM, opsonic function of plasma may be reduced.

BIOCHEMISTRY OF MALNUTRITION

In 22 years since the classic treatise 'kwashiorkor' appeared (Trowell, Davies and Dean, 1954) there has been a significant advancement in the understanding of the alteration in the biochemistry and physiology of PEM. During this period, emphasis has shifted from the description of biochemical findings to a description of functional change and its control mechanisms. It had to be realized that these changes could not be regarded simply as impaired or disturbed functions, and Waterlow's (1968) concept of an adaptation in the child to an

environment ranging from hostile to suboptimal has been a major contribution to the understanding of nutrition and malnutrition.

1. Protein and amino acid metabolism :

In the child or infant with moderate to severe PEM total body protein/albumin is reduced. Some organs are much more affected. Plasma protein is reduced in kwashiorkor, the greatest reduction occuring in the albumin fraction (Waterlow, Cravioto and Stephen, 1960).

Muscle fat in marasmus is more severely depleted (Standard, Wills and Waterlow, 1959; Garrow, Fletcher and Halliday, 1965; Halliday, 1966, 1967; Waterlow and Alleyne, 1971).

In kwashiorkor, albumin fraction was reduced as observed by various workers (Waterlow and Alleyne, 1971; Whitehead and Alleyne, 1972).

(i) Albumin :- It is now well established that the catabolic rate of albumin in severe PEM is reduced to about half the rate found after recovery (Cohen and Hansen, 1962).

In a study, James and Hay (1968) established that with a low protein diet the rate of synthesis was lower in malnourished children than in the recovered child.

Albumin synthesis rate rose and fell promptly when protein intake was increased or decreased and these changes were more marked in the malnourished than in the recovered child. It was concluded from the studies that a reduction in protein intake was followed rapidly by a decrease in the synthesis rate of albumin and catabolic rate was not directly affected by dietary protein intake nor by the rate of synthesis or plasma concentration of albumin.

In cases of protein energy malnutrition, total serum proteins particularly albumin and transferrin were significantly decreased. This has been attributed to the increased antigenic challenge of chronic infections in malnourished children (Cohen and Hansen, 1962; Alvarado and Luthringer, 1971).

Similar findings as have been reported in other children who suffered from malnutrition (Jose and Welch, 1970). Hypoalbuminemia was more pronounced in malnourished infected children, reflecting their lower nutritional status. This also appeared to be reflected in lower intestinal albumin levels found in this group.

Serum albumin, total serum protein levels repeated two weeks after nutritional therapy showed increased mean serum levels of albumin and total serum proteins (Neuman et al, 1975).

Bell et al (1976) worked on immunoglobulin and albumin levels in PEM from Caucasian, Indonesian and Australian children. They found that there were decreased levels of serum albumin.

(ii) Globulins :- Gitlin et al (1958) in their study, noted a diminished rate of synthesis of albumin but not of gamma globulin in uncomplicated kwashiorkor. In kwashiorkor complicated by infection IgG synthesis was increased three-folds.

Cohen and Hansen (1962) also noted that gamma-globulin metabolism, unlike that of albumin, was unaffected by nutritional status and in the presence of infection the synthesis rate was greatly increased.

(iii) Total body protein turn-over :- The effect of diet on protein turn-over had been studied in rats and in malnourished children. Waterlow and Stephen (1967, 1968) in their study on rats concluded that protein turn-over did not reduce by short periods of starvation or protein deprivation but a 30% reduction occurred after 5-6 weeks on low protein diet.

Waterlow (1975) and Young et al (1975) have shown that the rate of overall protein synthesis decreased with age.

(iv) Protein turnover in different tissue :- Protein synthesis in the liver was well maintained, while it was greatly reduced in muscle when a low protein diet or amino acid deficient diet was fed to rats for 3 days (Millward, 1970; Millward and Garlick, 1972).

(v) Amino acid metabolism :- In severe PEM total plasma amino acid concentration was reduced to one-half the normal value. In kwashiorkor there was a fall in the plasma concentration of most of the essential amino acids. Marked reduction was seen in the case of branched-chain amino acids and threonine, while lysine and phenyl alanine was less affected. The plasma concentration of non-essential amino acids was fairly well maintained or even increased (Arroyave et al, 1962; Holt et al, 1963).

2. Lipid Metabolism :

Fatty liver was a striking feature in kwashiorkor and was described by Williams (1933). Chemical determination of liver lipid at post-mortem had shown a severe degree of fatty infiltration of the liver in kwashiorkor even in the absence of hepatomegaly (Waterlow, Bras and Depass, 1957).

Mechanism of fatty lever :- Severe fatty infiltration leading to hepatomegaly carries poor prognosis and fat content in the liver of 40% was associated with high mortality (Waterlow, Cravioto and Stephen, 1960).

In fatty liver, the liver size increased, became pale in colour and firmer in consistency. Glycogen levels increased in the liver cells in kwashiorkor (Waterlow & Weisz, 1956; Stuart et al, 1958).

Fat accumulation was less severe or even absent in children in marasmic type of malnutrition (Chowdhuri, Bhattacharya and Basu, 1961). Hepatomegaly due to fatty liver was more constantly associated with fatal outcome in kwashiorkor. In Jamaica a high degree of oedema was also observed (Montgomery, 1963).

Excess of cholesterol in vitro altered the lipid composition of lymphocytes and granulocyte membrane and impaired their function, led to increased susceptibility to certain infections. Large amounts of fatty acid inhibited in-vivo primary and secondary response to certain antigens and in-vivo lymphocyte response to mitogens. Reticulo-endothelial system function was inhibited, granulocyte migration and bactericidal capacity was impaired.

Excess of polyunsaturated fatty acid supressed granulocyte function and delayed hyper-sensitivity reaction. Increased glycogen levels in liver cells were also associated with reduced levels of glucose-6-phosphatase (G6PD) activity (Fletcher, 1966).

Most of the available evidence supports the idea that liver has a reduced ability to dispose triglycerides which therefore accumulates and gives rise to fatty liver. The low fasting level of serum triglycerides in untreated case, the fact that the liver fat was virtually all triglyceride and that marked rise in serum triglyceride accompanied deffating of the liver when treatment begun, was all consistent with this theory. Very low density lipoproteins (d $\angle$ 1.063) equivalent to pre-beta fraction, is thought to be responsible for the transport of fat from the liver to plasma (Flores et al, 1970).

Plasma lipoprotein had important immunoregulation affect on cholesterol and high density lipoproteins essential for lymphocyte function. However, excess amount was immuno-suppressive. Very low density lipoprotein specifically inhibited protein and DNA synthesis in many cells and lymphocytes.

There was also a possibility of increased hepatitis antigen in kwashiorkor (Suskind and Olson, 1973). Laboratory animals deprived of fatty acid showed lymphoid atrophy and reduced antibody responses both to T-dependent and T -independent antigen (Newbarne et al, 1981).

3. Body fluid :

(i) Total body water :- A study of J. Patrick, Reeds, Jackson and Ricou (1957 Unpublished) showed that TBW % was the same before and after recovery of PEM. Garrow, Smith and Ward (1968) concluded there was over hydration in both kwashiorkor and marasmus and that an expansion of the extra-cellular fluid space accounted for most part of the increase in total body water.

(ii) Extracellular water :- has been estimated in vivo in PEM by measuring the distribution space of thiocynate, thiosulphate (Gollan, 1948; Kerpel-Fronius and Kovach, 1948; Brinkman et al, 1965; McLaren and Pellett, 1970) or bromide (Alleyne, 1967; Graham et al, 1969).

(iii) Intracellular water :- was determined indirectly by substracting extracellylar water from the total body water and result obtained were therefore open to some criticism. Intracellular water was lost in patients suffering from kwashiorkor and that during recovery there was an absolute increase in intracellular water, presumably due to shift of water into intracellular compartment (Brinkman and Hansen, 1963).

(iv) Oedema :- Hypoproteinaemia predisposes to oedema. Montgomery (1963) demonstrated a significant correlation of hypoproteinaemia with the degree of oedema.

Srikantia (1968) has proposed that an increased activity of anti-diuratic hormone (ADH) played an important role in the formation of oedema in kwashiorkor. He postulated that there was defective inactivation of ADH due to structural and functional changes in the liver, which also promoted release of ferritin into plasma. Srikantia (1968) has reported the presence of ferritin in the plasma in kwashiorkor and in nutritional oedema in adults, but not in the plasma of marasmic infants or normal control.

Increase in aldosterone secretion has also been postulated as a causative factor of oedema in kwashiorkor. But there was no good evidence that increased aldosterone concentration or secretion rate was responsible for the oedema in kwashiorkor (Migeon et al, 1973).

Albumin affected plasma colloidal osmotic pressure. Colloidal pressure did not fall significantly until albumin concentration fell to between 25.1 and 27.5 gm/litre (Coward, 1975). An increase in serum globulin concentration resulting from infection explains that colloidal osmatic pressure was maintained despite an initial fall in plasma albumin concentration.

Halliday (1967) in his data on whole body analysis, did not support the suggestion of Frank et al(1975)

that preservation of subcutaneous fat was necessary for the development of pitting oedema.

4. Skin and Hair change :

Skin gets depigmented and in very severe cases, is covered with darker shiny patches which then crack like patched earth and has been described by William (1933) as 'crazy pavement'. The cracks between the dark patches remain infected and desquamated followed by ulceration particularly in the folds and crease of axillae, groins and other area.

The hair looses its lusture and natural curls. Colour changes of hair in dark haired races occur towards brown, red, blond and to near white. The changes start at the hair line and could affect separate segments of length of each hair to give the 'flag sign' (Chauarria, 1953). Brittleness, thinness and sparseness of hair was accompanied by easy 'pluckability'. The hair changes, particularly the pigmentation, were probably more related to the duration of malnutrition and could be completely absent in acute protein energy malnutrition.

T and B cell count :

T and B lymphocytes can be identified by various methods. Human B cell possess surface immunoglobulins detectable by direct immunofluorescence. They also

possess receptors for aggregated immunoglobulins, for antigen antibody complexes and for third component of complement. It is believed that rosette is formed by rapid release of metabolised receptor substances on the living cell-surface. These receptor substances are possibly positive bivalent ions since ethylene diamine tetractic acid will block the rosette formation (Jondal, 1972). These receptors are detected by erythrocytes coated with antibody or complement that surrounds B lymphocyte in a cluster (Wybran and Fundenberg, 1973).

Presently, the spontaneous formation of rosette with sheep erythrocyte appears to be a specific property of T lymphocytes and membrane immunoglobulin detectable by immunofluorescence constitutes the most reliable marker of B cells (Saligman, 1974). This method of identifying lumen T cells by directing antibodies against T & B lymphocytes and these rosette formation was described by Fundenberg (1975). However, the fundamental nature of rosettes formation is still not known.

Comparable results using either ethyline diamine tetra acetic acid (EDTA) or heparin (an anti-coagulant) for rosette testing have been reported. Handfield et al (1975) and Fairbank (1976) reported that as the concentration of heparin was increased in the test system, the percentage of T lymphocyte rosette formation decreased. Normally

there are more than 1500 circulating T cells/mm^3 each being less than 10 u in diameter. In some cases of T cell deficiency, number of lymphocyte count is normal or even elevated but the lymphocyte are larger than 10 u in diameter. Monocytosis and eosinophillia are commonly associated with T cell deficiency (Buckley et al, 1972; Rose and Friedman, 1976).

Steel et al (1974), Chisholm et al (1976) noted that T and B lymphocyte rosette formation is affected by temperature, incubation time and red cell to lymphocyte ratio. The data obtained indicate that the SRBC/lymphocyte ratio is a primary variable in determining the percentage of T cell rosette formation.

Variation of lymphocyte count with age and sex :

Zochorski and co-workers (1971) noted that there was no significant variation of lymphocyte count with age and sex.

Nybran et al (1972) found that there was no difference in T and B cell counts of infant and children. Wasler and Hutteroth (1974) found no difference in total lymphocytes and relative number of T lymphocytes in the peripheral blood of young children and adult individuals.

In most of the studies regarding the deviation of T lymphocyte and B lymphocyte counts in disease,

age characteristics of the control data have not been given, though Elhilali and associates (1978) have emphasized the importance of using age matched control.

Normal distribution of T and B lymphocytes :

Fleisher et al (1975) studied the sub-population of T and B lymphocytes in the peripheral blood of children and adult using E and EAC rosette assays. Children under 18 months of age were found to have decreased percentage of E-binding(T) lymphocytes and an increased percentage of EAC binding (B) lymphocytes as compared to older children (18 months to 10 years) and adults. The absolute number of E-binding and EAC binding lymphocytes was increased in children under 18 months of age.

Neighburger et al (1976) studied the distribution of T and B lymphocytes in peripheral blood of children and adult. They found the following distribution.

	T cell %	B cell %
Children	44.0 ± 4.2	30.4 ± 3.1
Adult	46.3 ± 1.8	26.5 ± 2.3

Age group	T (E-binding cell) Percentage Mean ± S.D.	Range	Absolute number Mean ± S.D.	Range
< 18 months	50.2 ± 8.7	33 - 67	2970 ± 690	1620-4320
18 months - 10 years	56.8 ± 5.9	45 - 69	1840 ± 640	59-3090
Adult	64.0 ± 6.9	51 - 78	1910 ± 590	750-3070
	B (EAC binding) cell			
< 18 months	26.2 ± 6.3	14 - 39	1530 ± 540	470-2590
18 months - 10 years	22.7 ± 3.4	16 - 29	720 ± 280	170-1270
Adult (> 10 years)	17.2 ± 3.9	11 - 23	540 ± 170	170-510

Variation of total average rosetting values and range for adult control and PCM children :

Chandra et al (1972) observed that in PEM T lymphocyte rosetting is decreases while B lymphocyte rosetting value remains unaffected (except in children who accuired infection in whom it is increased 3 folds). Betsy and Bang et al (1975) also supported the finding that T lymphocyte rosetting was decreased in PCM.

Prabha et al (1978) reported reduction of absolute number of T and B lymphocytes in blood, thymus, lymph nodes and spleen in PCM.

PCM type	Degree	Average % B	Average % T
K	+++	21	34
K	++		
	+	29	43
M	+++	33	44
	++		
Kw (Nutritional oedema)	+++	51	53
	++		
Average PCM range		36 13-39	46 23-63
Average adult control		31 22-38	63 54-72

Averaged B rosetting values in children with PCM, with and without clinical infection at the time of admission

Average B cells
(36%)

(Uninfected) 26% (13-30%)

43% (infected) (30-39%)

Variation of serum albumin in infants and children with PCM alongwith control values :

Protein	Normal value	Severely malnourished			Moderately malnourished	Control
		Range	Kwashiorkor	Marasmus		
Albumin	7 2.5 gm/ 100 ml.	2.4 ± 1.3	2.0 ± 0.1	3.4 ± 0.2	3.9 ± 0.2	4.3 ± 0.9

Ferghsson and co-workers (1974) observed that children with low T cell percentage had severe hypoalbuminemia (⩽ 2 gm/100 ml) as well.

Change in Hb% and lymphocyte count in PCM and control :

Normal value	Severely malnourished	Moderately malnourished	Control
	% of deficiency	% of deficiency	% of deficiency
Haemoglobin 7 10.0 gm/ 100 ml.	60.0%	17.5%	12.5%
Lymphocyte count 600 - 4,500	2860 ± 300 (9%)	3380 ± 240 (5%)	3270 ± 290 (2%)

All these changes were reported by Neumann et al (1975) in their studies.

Delayed hypersensitivity in Protein Energy Malnutrition :

In vitro T-lymphocyte function can be measured by delayed hypersensitivity reaction using a variety of antigens to which majority of older children and adults have been sensitized. Generally, useful skin test antigens are 1 : 100 dilution of tetanus toxoid, protein purified derivative (PPD), histoplasmin, mumps virus, extract of candida, Trichophyton, phytohaemagglutinin (PHA) and Dinitrochlorobenzene (DNCB).

Epstein and Kligman (1959) reported that a single application of DNCB sensitized over 90% of a large series of normal control, the ability to initiate and manifest DNCB hypersensitivity was not predictive of any form of morbidity in either age group.

Eisenberg (1962) in his study of DNCB test using 1000 ug/0.1 ml concentration of DNCB for sensitizing dose and 50 ug/0.1 ml for challange dose reaction was read day 2, observed severe itching present at the site and also vesiculation, which was less common. He opined that both indicated sensitization. Reaction was graded as follows :

3+ spontaneous flare occurring at both sensitizing and challange dose sites.

2+ spontaneous flare occurring only at sensitizing dose sites.

1+ absence of spontaneous flare but reapplication of challange dose causing an equivocal delayed hypersensitivity reaction.

-ve No reaction. No spontaneous flare occurring even after reapplication of challange dose.

Depression of CMI may partly determine the pattern of infection in PCM. Various postulates have been put forward by Scrimshaw et al (1959) with emphasis on the effect of specific nutritional deficiency on various virus and bacterial growth. In some cases the germinal centres were so depleted that depression of humoral immunity might also have been expected.

Poor reaction denote depression of the CMI. Depression of CMI has been postulated in PCM to explain negative DNCB reaction (Harland, 1965).

Depression of CMI in PCM could be the result of an absolute or relative deficiency of amino-acid for cell multiplication. Alternatively, children with PCM are known to have raised level of plasma cortisol (Rao et al, 1966; Alleyne, 1966; Rao et al, 1968), which can depress the thymo-lymphatic system.

Smith et al (1971) reported that in the PCM group, 12 (70%) had no reaction to DNCB and 5 (30%) had a grade I reaction, whereas in the control group, none failed to react,

6 (32%) had grade I, and 13 (68%) grade II (++) reaction. The response to chemical substances was significantly less in PCM group ($P < 0.01$). A significantly greater loss of germinal center was found in all cases with PCM, with the greatest loss in kwashiorkor group.

Malnourished children have impaired immunocompetence (Smythe et al, 1971; Chandra, 1972; Seth and Chandra, 1972) and cell mediated immunity is consistently depressed.

T cell undergo mitosis in response to stimulation in vitro by specific antigen or by mitogen (plant derived material) that perturbs the lymphocyte membrane and trigger's cell division (Smythe et al, 1971). Skin testing is an important test for the assessment of cell mediated immunity. Catalone et al (1972) developed a method for DNCB contact sensitization which response to antigen is evaluated by an experienced observer by noting induration, erythma, oedema, as well as size of reaction. Positive test has a value in establishing the presence of normal T cell function but negative skin test is considered as inconclusive evidence of deficiant T cell function, particularly in young children with limited antigen experience. Direct sensitization with 2, 4 dinitrochlorobenzene can be performed followed by a subsequent challange of cutaneous reactivity with the same material.

The capacity to become sensitized to a new antigen may be tested by application of DNCB to the skin, followed at 2 week later by patch testing at different sites with the same material. Thus ability of an individual to develop cell mediated immunity denote can be determined by applying directly to the skin, a chemical DNCB to which individual had not been previously exposed. The chemical combines with skin protein to form an immunogenic substance that stimulates sensitization of T cell to DNCB. The reapplication of DNCB on skin if CMI is intact. DNCB skin test for the assessment of cellular immunity has advantage over other intradermal tests. Reliance upon previous exposure to allergen is unnecessary and circulating antibody does not develop with contact sensitization which renders it more exact test for testing cellular immunity.

Schlesinger and Stekel (1974) carried out DNCB skin testing in malnourished, healthy and infected patients. They observed that skin test response was depressed in malnourished children in comparison to healthy control. But, infected patients as compared to well nourished infant showed intense positive reaction with DNCB.

Bang et al (1975) confirmed that DNCB was generally to be considered as a measure of cell mediated immune response. He also observed that kwashiorkor had

lower T lymphocyte rosetting values than in case of other types of PCM. Children with kwashiorkor seemed to have more disabilities.

Watts (1980) postulated that in malnourished children, atrophied thymus was the most likely cause for impaired cell mediated immune response. Author further observed that DNCB test could also be used in children before subjecting them to immunization procedure, when a sensitizing dose was applied, the immediate reaction produced expressed a general inflammatory response. Sensitizing dose was expressed by the occurrence of a spontaneous flare 7 to 14 days later, or by reaction to a challange dose of DNCB. Equivocal reactions required histologic examination.

Study of Sanjeev et al (1981) revealed that malnourished children developed impaired reaction to DNCB (45.9%). They compared DNCB reaction in control and malnourished children and reported his observation as follows :

DNCB reaction	Control group		Study group	
	No.	%	No.	%
+ 3	11	36.7	31	18.2
+ 2	12	40.0	49	28.9
+ 1	3	10.0	12	7.0
-ve	4	13.3	78	45.9
Total	30	100.0	170	100.0

MATERIAL AND METHODS

MATERIAL AND METHODS

The present study was conducted in the department of Paediatrics, M.L.B. Medical College, Jhansi, between November 1988 to December 1989.

Selection of cases : Children suffering from protein energy malnutrition between the age of 6 months to 5 years, attending Paediatrics out patient department and those admitted in Paediatrics ward were selected for the study. The diagnosis of Protein Energy Malnutrition was based on present weight (weight below 70% of the 50th centile of NCHS standard for age) history and clinical examination. Suspected cases of childhood tuberculosis, those taking steroids or suffering from any allergic disorder were excluded from the study. Children on immuno-suppressive drugs and those suffering from immune deficiency disorder were not considered for the study.

1. Healthy normal control :

Healthy children (6 months - 5 years) whose weight-for-age was more than 80% of 50th percentile of NCHS standard were considered for inclusion in the present study.

2. Malnourished children :

Malnourished cases were further grouped on the basis of McLaren Classification (1967). Besides name, age, sex, address and socio-economic status, following facts were recorded in each case.

i) Present, past and family history :- From parents or other family members detailed history was obtained regarding illness, in a chronological order. Emphasis was given to elicit the history of chronic diarrhoea, worm infestation, failure to thrive, fever, cough and vomiting. An enquiry was made about the definite history of tuberculosis in parents, sibling, near relatives and neighbours.

ii) Physical examination :- A thorough clinical examination was done to observe psychomotor change, pallor, oedema, skin and hair changes, status of subcutaneous tissue and muscle mass. Eyes were examined for any evidence of xerosis. Skin lesion like hypo-pigmentation/ hyper-pigmentation, hyper-keratosis or any other dermatosis was noted. Examination of lips, gums and tongue was carried in each case to detect the presence, if any, of angular stomatitis, cheilosis, sponginess and glossitis respectively. Skeletal system and thyroid gland was examined for abnormalities.

iii) Dietary history :- Dietary history was recorded with special emphasis on the following points :

(a) Age upto which breast milk was given.

(b) Age at which artificial milk was started, type of artificial milk and quantum of dilution used in the milk formula.

(c) Age at which semi-solid and solids were started.

(d) Present calorie and protein consumption of the child and also his past calorie and protein consumption were assessed.

iv) Immunization status :- History of immunization was recorded from the parents or family members. Also, left upper arm of the child was examined for any scar mark pertaining to BCG vaccination, given in the past.

v) Antenatal, natal and postnatal history :- History relating to intake of drugs in the mother and also the type and mode of delivery, condition of baby after birth was taken, to rule out the secondary factors which give rise to malnutrition.

vi) Developmental history :- History of developmental mile-stones achieved in the fields of gross motor, fine motor, social and speech was recorded to rule out the presence of mental retardation and cerebral palsy, as the causative factors of malnutrition.

vii) Anthropometric examination :

- Weight :- It was recorded nearest to 0.05 kg by infant weighing machine for infants weighing less than 10 kg, while adult type weighing machine with an accuracy of 0.5 kg was used for cases weighing more than 10 kg.

- Crown-heel length :- It was recorded nearest to 0.1 cm. by an Infantometer, for all patients upto the age of 5 years.

viii) Laboratory techniques :

Material used -

- Heparin (Preservative free)
- Minimum essential medium (MEM) - Eagle
- Alsever's solution
- Phosphate buffer saline (PBS)
- Pooled normal human serum
- Anti-sheep haemolysin (Inbocuptor)
- Methyline blue 0.2%.

Alsever's solution :

Glucose	:	24.6 gm
Trisodium citrate(dehycrate)	:	9.6 gm
Nacl	:	5.04 gm
Distilled water	:	1200 ml

pH of Alsever's solution was adjusted to 6.1 with 10% citric acid. Solution was sterilised by low pressure autoclaving and stored in a refrigerator.

Phosphate buffer saline (PBS)

A) 0.15 M - $Na_2 HPO_4 2H_2O$ (23.4 gm/litre)

B) 0.15 M - $Na_2 PO_4$ 21.3 gm/litre

C) Normal saline - 9.0 gm Nacl/litre.

For phosphate buffer saline (PH 7.4) solution A (18 ml) was mixed with solution B (82 ml) and then solution C (100 ml) was added. The solution was then sterilized by low pressure autocleving and stored in a refrigerator.

Pooled normal human serum :- Venous blood was drawn asceptically into clean and dry test tubes 15 ml each, from 4 persons. Test tubes were incubated in water bath at 37°C for 30 minutes and then at 40°C for 120 minutes. The clot from each tube was removed gently with a glass rod and the tubes were centrifuged. The clear serum from each of the tubes was collected and mixed with each other. Pool d serum was stored at -20°C. Small aliquotes were used only once after thawing.

Collection of blood sample :- 10 ml heparinised peripheral blood sample (25 unit of heparin/ml of blood) was collected in the sterile tube from each patient for T and B cell counts. Also the venous blood was simultaneously collected in double oxalate vial from the patient, for total and differential leukocyte counts.

Total leukocyte count (TLC) :- One in 20 dilution of blood war made by adding 0.02 ml blood to 0.38 ml of WBC diluting fluid (Turk's fluid) in 7.5 x 10 mm test tube. The suspension was mixed by gentle tilting and rotating by hand for 2 minutes. The Neubaur's counting chamber was charged with suspension and viewed with 5 mm objective under a microscope. The number of leukocyte were counted and calculated as below :

$$TLC = N \times 200/cu\ mm.$$

'N' is number of leukocyte counted in each mm square area. Total four squares was counted, in which each large square contain 5 small square (total 80 small square will count).

Differential leukocyte count (DLC) :- A thin and uniformly prepared peripheral blood smear was stained for 8 to 10 minutes with Leishman stain washed with buffered solution (pH 6.3) containing $K H_2 PO_4$, 9.19 m/l & $Na_2 HPO_4$, 9.5 m/l (mixed together in the ratio of 1.03 : 1). The slide was then dried in air. Leukocytes were counted using oil emersion lens and the percent distribution of different leukocytes was calculated after counting 200 cells.

Absolute lymphocyte count (ALC) :- Absolute count was calculated in every case from the total and differential leukocyte count using the following formula

$$ALC = \frac{TLC \times \%\ lymphocytes}{100}$$

Evaluation of T and B lymphocyte :

1. Preparation of lymphocyte rich plasma :

The lymphocytes were separated from the heparinised peripheral blood by gravity sedimentation method. 10 ml of heparinised blood (25 unit/ml blood) collected in a sterilized test tube was kept up-right at room temperature for one hour. The leukocyte-rich plasma was collected and centrifuged at 1000 rpm for 15 minutes. The clear plasma was separated and the cell button was suspended in phosphate buffer saline PBS/MEM minimal essential medium. The concentration of lymphocyte suspension was adjusted to 2 - 3 x 10^6 per ml in PBS/MEM.

2. Preparation of sheep RBC solution :

Sheep blood collected in equal volume of Alsever's solution was stored in a refrigerator for 3 - 5 days and thereafter used upto 14 days. Sheep blood was washed thrice with buffer saline. One volume of packed cells was suspended in 18 volumes of buffer saline to give a slightly greater concentration than 5% suspension. One ml of this suspension was lysed with exactly 14 ml of distilled water and optical density (OD) was measured at 540 ohm with distilled water as blank. A lysate with optical density of 0.7 represented 5% concentration or 1 x 10^9 cell/ml. From the O.D. of sample tested and volume of the suspension (vi), the final volume (vf) was calculated according to the relationship :

$$vf = \frac{vi \times O.D.}{0.7}$$

Finally suspension was adjusted to make a standard solution of sheep RBC.

Demonstration of T cell by sheep RBC Rosette (E Rosette) :

Sheep RBC's were washed thrice with PBS and 0.5% suspension was made in M.E.M. solution. Lymphocyte count was adjusted to 2-3 x 10^6 per ml in RBC. To 0.5 ml of sheep RBC suspension was added 0.5 ml of lymphocyte suspension in PBS and mixture was incubated for 15 minutes at 37°C in water bath. After centrifugation for 5 minutes at 500 rpm, mixture was incubated at 4°C over night. Supernatant was removed and pellet was resuspended in M&M (2-3 drops). Finally wet preparation was made and stained with methylene blue (0.2%) and 200 rosette forming cells were counted under a microscope to calculate the percentage of rosette forming cells.

When three or more SRBC's were seen adhering to a lymphocyte, it was considered as a rosette forming cell. The absolute T cell count was calculated as follows -

$$\text{Absolute T cell count} = \frac{\text{ALC} \times \% \text{ T cells}}{100}$$

Demonstration of B cells by forming EAC rosette (Fleisher et.al., 1975 and Shevach et.al., 1972) :

B lymphocytes in normal peripheral blood can be identified by the presence of at least three surface marker,

receptors for modified components of complement, surface immunoglobulins and receptors for aggregated IgG. Complement receptor bearing lymphocytes can be detected by the binding of antigen, Erythrocyte (E), antibody (A) and complement (C) to form EAC rosettes.

To 0.5 ml of 5% of SRBC suspension, 0.5 ml of anti-sheep haemolysin in appropriate dilution (1 : 400 assessed earlier) was added and incubated for 15 minutes at 37°C. After washing three times with phosphate buffer saline and resuspending in PBS and thereafter adding 0.5 ml of 1 : 10 diluted complement (Human serum), tube was incubated for 45 minutes at 37°C. The cells were washed with phosphate buffer saline and then resuspend to make a concentration of 0.5% of EAC in phosphate buffer saline.

To 0.5 ml suspension of lymphocyte ($2-5 \times 10^6$ ml), 0.5 ml of EAC in PBS was added and incubated at 37°C for 30 minutes. The solution was resuspend and wet preparation was prepared and stained with 0.2% Methylene blue. Finally 200 cells were counted under the microscope to calculate the percentage of EAC rosette forming lymphocytes.

A group of three or more SRBC's adherent to a lymphocyte was considered as EAC rosette. Absolute B cell count was calculated as follows :

$$\text{Absolute B cell count} = \frac{\text{ALC} \times \% \text{ EAC rosettes}}{100}$$

2,4 Dinitrochlorobenzene (DNCB) contact sensitization test :

Stock solution of DNCB in acetone of 1000 ug/0.1 ml and 50 ug/0.1 ml concentration was made and stored in an amber coloured bottle at room temperature. This solution was changed after every 3 months. Stainless steel ring of 2 cm diameter was placed at the site of application of DNCB so that fixed area was obtained. Sensitizing dose of 1000 ug/0.1 ml was applied on the right upper arm on voler surface slightly towards the medial side. Simultaneously a challange dose of DNCB (50 ug/0.1 ml) was applied on the medial aspect of the flexor side of right forearm.

After the application of DNCB these sites were covered and the subject's parents were instructed not to wash the site for 24 hours. Site was examined after 48 hours for a spontaneous flare, indicated by the appearance of erythema, induration and vesiculation.

Reaction was graded according to the criteria proposed by Eisenbery (1962) :

4+ unequivocal spontaneous flare occuring at both sensitizing and the challange dose site.

3+ A spontaneous flare occuring at only the sensitizing dose site.

2. In the absence of a spontaneous flare, reapplication of a challange dose elicited an unequivocal reaction.

If no spontaneous flare occurred even after the reapplication of challange dose, the reaction was considered as negative.

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OBSERVATIONS

OBSERVATIONS

The present study was conducted in the department of Paediatrics, M.L.B. Medical College, Jhansi, over a period of 1 year from January 1989 to December 1989. The aim of the study was to assess the immunological status of children suffering from Protein Calorie Malnutrition (PCM).

A sample of 30 children aged between 1 to 5 years was taken for this study which included 9 healthy children and 21 children suffering from protein calorie malnutrition.

Blood haemoglobin and serum albumin levels were estimated and anthropometric measurements viz. weight and height/length were noted in each case.

Humoral immunity was assessed by EAC rosette (B cell) count. Cellular immunity was assessed by E rosette (T cell) count and dinitrochlorobenzene skin sensitization test in every child of the study and control groups.

1. Classification of PCM cases :

Malnourished cases were classified according to age independent and age dependent criteria.

According to age independent criterion (McLaren Classification, 1967) malnourished cases were sub-divided into three groups viz., Marasmus, Kwashiorkor and Marasmic-Kwashiorkor group. Based on this classification, 13 cases were suffering from Marasmus, 3 had Kwashiorkor and 5 cases had Marasmic-Kwashiorkor (Table I).

Table I

Distribution of children of study group according to clinical classification (McLaren Classification, 1967).

Clinical group	McLaren score	No.of cases
A. Control group	7 15	9
B. Study group :		
Marasmus	0 - 3	13
Kwashiorkor	4 - 8	3
Marasmic-kwashiorkor	9 - 15	5
Total		30

The study group was also sub-divided according to the classification of Nutritional Sub-committee of Indian Academy of Paediatrics (1972). The distribution of cases in various grades of malnutrition was as shown in table II.

In the present study there was no case falling in grade I malnutrition. Three cases had grade II and 8 cases had grade III malnutrition. There was 10 cases of grade IV malnutrition in the present study.

Table II

Distribution of cases according to IAP classification.

Grade of malnutrition	Range of expected weight	No.of cases
A. Control group	7 80%	9
B. Study group :		
Grade I	71 - 80%	-
Grade II	61 - 70%	3
Grade III	51 - 60%	8
Grade IV	< 50%	10
Total		30

2. Clinical features in Protein Calorie Malnutrition cases :

Table III shows the clinical features observed in malnourished cases. There were 15 (71.4%) cases of PCM who had chronic diarrhoea. Oedema and hair changes were seen in 3 (14.3%) cases each, while hepatomegaly and skin changes were observed in 5 (24%) and 8 (38%) malnourished cases respectively.

Table III

Some of the presenting clinical features in Protein Calorie Malnutrition cases (N = 21).

Clinical feature	No.of cases	Percentage
Chronic diarrhoea	15	71.4
Oedma	3	14.3
Hair changes	3	14.3
Skin changes	5	24.0
Hepatomegaly	8	38.0

The malnourished cases were further assessed with respect to the frequency of clinical manifestations viz., diarrhoea, oedema, hair and skin changes occurring in various grades of malnutrition. Most clinical manifestations occurred with greater frequency in severe grades of malnutrition, as observed in the present study (Table IV).

Table IV

Showing the distribution of clinical manifestations among various grades of malnutrition.

Clinical group (IAP Classification)	No.of cases	Number (percentage of cases				
		Diarrhoea	Oedema	Hair change	Skin change	Hepatomegaly
I	-	-	-	-	-	-
II	3	1 (33.33)	-	-	-	-
III	8	5 (62.5)	1 (12.5)	1 (12.5)	2 (25.0)	4 (50.0)
IV	10	9 (90.0)	2 (20.0)	2 (20.0)	3 (30.0)	4 (40.0)
Total	21	15 (71.4)	3 (14.3)	3 (14.3)	5 (24.0)	8 (38.0)

3. Age distribution in control and study group :

The age distribution of cases suffering from malnutrition is shown in table V. Out of a total of 21 malnourished cases examined, maximum number of cases were in 1 - 2 years and 2 - 3 years age groups (14 and 5 cases respectively). In 3 - 4 years and 4 - 5 years age groups, sample consisted of only one case each.

In the control group, 2 cases each were sampled in 1 - 2, 2 - 3 and 4 - 5 years age groups. There were 3 cases in 4 - 5 years age group.

Table V

Age distribution of control and study groups.

Clinical group	Age (years) 1-2	2-3	3-4	4-5	Total cases
Control group	2	2	2	3	9
Study group					
I	-	-	-	-	-
II	1	2	-	-	3
III	4 (14)	2 (5)	1 (1)	1 (1)	8
IV	9	1	-	-	10
Total	16	7	3	4	30

4. Weight/Height^2 ratio in the assessment of protein calorie malnutrition :

It is evident from table VI that mean weight/Height^2 ratio was 0.00154 in the control group, while this ratio was 0.00135, 0.0009 and 0.0009 in grade II, III and IV PCM groups respectively (there was no case falling in grade I malnutrition). A gradual fall of weight/height^2 ratio was observed as the severity of malnutrition increased.

Table VI

Mean weight/height^2 ratio in different groups of malnourished cases and the control group (Rao et al, 1970).

Groups	Total No.of cases	Mean weight/height^2
Control	9	0.00154
Study group :		
Grade I	-	-
Grade II	3	0.00135
Grade III	8	0.0009
Grade IV	10	0.0009

Table VII shows that 7 out of 9 cases of control group had mean weight/height ratio above 0.0015 while 2 cases had a mean value below 0.0015. In the study group out of 21 cases, 20 (95.2%) had a value below 0.0015 and only one (4.8%) had value above 0.0015. The weight/height^2 ratio was found to have sensitivity and specificity of 95.2% and 77.8% respectively.

Table VII

Distribution of cases according to the ratio of weight/height^2.

Groups	Total No.of cases	Mean weight/height^2 ratio			
		0.0015 & above		Below 0.0015	
		No.	%	No.	%
Control	9 (70%)	7	77.78	2	22.22
PCM					
Grade I -					
Grade II 3					
Grade III 8	21 (30%)	1	4.8	20	95.2
Grade IV 10					

Sensitivity : 95.2%

Specificity : 77.8%

5. Serum albumin in control and study groups :

It is clear from table VIII that value of serum albumin in control cases was much higher and statistically significant than the value observed in different grades of malnutrition.

In the control group 9 children had serum albumin ranging between 4.1 - 6.1 with a mean value of 4.53 ± 0.367 gm/dl. Children of grade II malnutrition (N = 3) had mean serum albumin of 3.71 ± 0.578 gm/dl and a range of 3.1 - 4.1 gm/dl, while in PCM of grade III mean value was 3.44 ± 0.112 gm/dl with a range of 3.0 - 3.6 gm/dl. Ten children belonging to grade IV had serum albumin range of 2.8 - 3.6 gm/dl and a mean value of 3.35 ± 0.534 gm/dl.

Fig. 1 shows that there was gradual decrease in serum albumin as the severity of malnutrition increased.

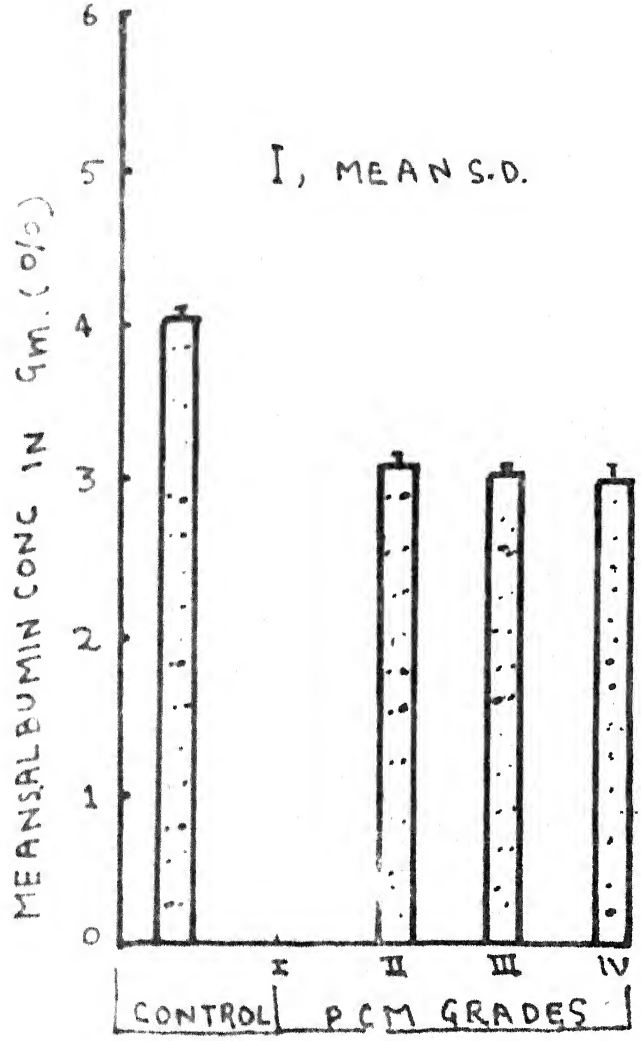


FIG-1 COMPRATIVE DIAGRAM SHOWING SERUM ALBUMIN CONC. IN CONTROL AND STUDY GROUP

Table VIII

Status of serum albumin (gm%) in control and study groups.

Clinical group	No.of cases	Range	Serum albumin level Mean	± S.D.
a. Control	9	4.1 - 6.1	4.53	± 0.367
b. Study group :				
Grade I	-	-	-	-
Grade II	3	3.1 - 4.1	3.71	± 0.578
Grade III	8	3.0 - 3.6	3.44	± 0.112
Grade IV	10	2.8 - 3.6	3.35	± 0.534

6. Haematological values in control and study groups :

Haemoglobin, absolute and differential leucocyte counts were done in each child. Haematological values of control and PCM groups were compared.

In the control group, mean Hb value was 12.16 ± 0.577 gm% while it was 11.4 ± 1.14 gm% in grade II, 11.0 ± 0.901 gm% in grade III and 10.4 ± 1.2 gm% in grade IV malnourished cases respectively.

Mean absolute lymphocyte count in control group of cases was 3777.50, while it was 3485.75, 3123.61 and 2496.22 in grade II, III and IV malnourished cases respectively.

Mean polymorph count in control group was 61.11% while in grade II, III and IV malnutrition it was 51.66%, 53.75% and 50.50% respectively.

Mean lymphocyte count in control group was 44.77 ± 0.941, while it was 40.33 ± 10.5, 38.5 ± 14.274 and 35.3 ± 12.129 in grade II, III and IV malnourished cases respectively.

It is evident from table IX that there was no significant difference in the polymorph counts of control and study groups. Values of haemoglobin, absolute lymphocyte count and mean lymphocyte count gradually fell as severity of malnutrition increased.

Table IX

Haematological values in control and study groups.

	Control group	Study group		
Haematological parameter	Mean ± S.D.	II Mean ± S.D.	III Mean ± S.D.	IV Mean ± S.D.
Absolute lymphocyte count	3775.50	3485.75	3123.6	2496.22
Hb. gm%	12.16 ± 0.577	11.4 ± 1.14	11.0 ± 0.901	10.4 ± 1.2
DLC				
Polymorph	61.11 ± 11.19	51.66 ± 12.05	53.75 ± 14.74	50.5 ± 11.90
Lymphocytes	44.77 ± 0.941	40.33 ± 10.5	38.5 ± 14.274	35.3 ± 12.129

7. Immunological status in control and study groups :

Immunological status was studied by observing the B-cell and T-cell lymphocyte counts in control and study groups.

B-Cell count (Table X and Fig. 2).

Mean of absolute B lymphocyte count in control cases was 696.804 ± 216.469. It was 812.45 ± 114.604 in grade II, 954.94 ± 531.09 in grade III and 1043.09 ± 377.64 in grade IV malnourished cases. Differences in absolute and percentage B-lymphocyte counts, between the control and various grades of malnourished cases were uniformly non-significant ($P > 0.05$).

In the control group, mean B-cell count was 27.66 ± 1.805%. Malnourished cases had a mean B-cell count of 27.33 ± 4.487%, for grade II, 25.87 ± 1.83% for grade III and 27.9 ± 1.79% for grade IV cases.

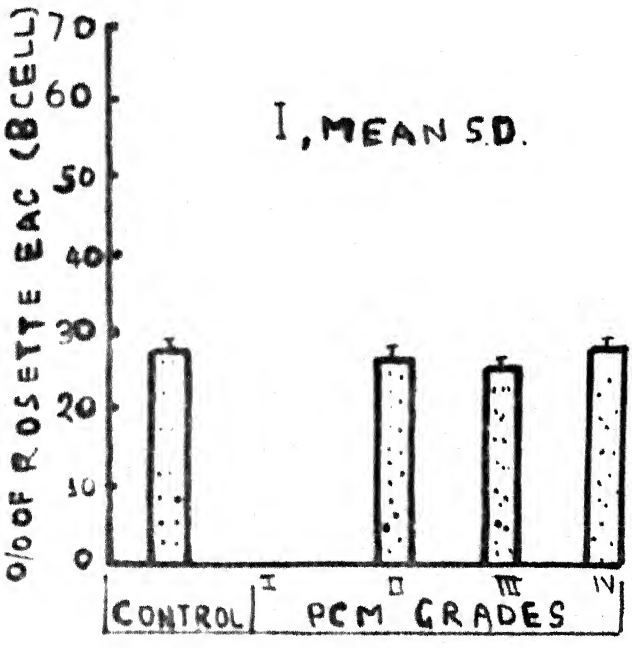


FIG-2 COMPRATIVE DIAGRAM OF EAC ROSETTE(%) IN CONTROL AND STUDY GROUP

Table X

Distribution of B-cell (EAC rosette count) in study and control group.

Groups	Control	Study			
		I	II	III	IV
No.of cases	9	-	3	8	10
Absolute B-cell count Mean ± S.D.	686.804 ± 216.469		812.45 ± 114.604	954.94 ± 531.09	1048.9 ± 377.64
Statistical difference	-		$P > 0.05$	$P > 0.05$	$P > 0.05$
B-cell % EAC rosette count Mean ± S.D.	27.66 ± 1.805		27.33 ± 4.497	25.87 ± 1.837	27.90 ± 1.790
Statistical difference	-		$P > 0.05$	$P > 0.05$	$P > 0.05$

$P < 0.05$ is significant

T-Cell count (Table XI and Fig. 3)

Absolute T-cell count for control group was 1240 ± 266.215, while it was 1105.15 ± 528.455 for grade II, 1350.80 ± 874.73 for grade III and 1122.76 ± 422.24 for grade IV cases of malnutrition. Statistically the difference in percentage T-cell count of control cases and grade II malnourished cases was non-significant ($P > 0.05$), while such a difference was significant when control cases were compared with grade III and IV cases. The statistical difference of mean absolute T-cell counts between control and grade II malnourished cases was not significant ($P > 0.05$). Such a difference between the control and grade III control and grade IV malnourished cases was significant ($P < 0.05$).

It is evident from table XI that mean T-cell count (E-rosette) in control cases was 52.0 ± 5.889%. In the study group, it was 33.33 ± 6.944% for patients of grade II, 30.5 ± 8.66% for patients of grade III and 28.1 ± 4.867% for patients of grade IV malnourished cases.

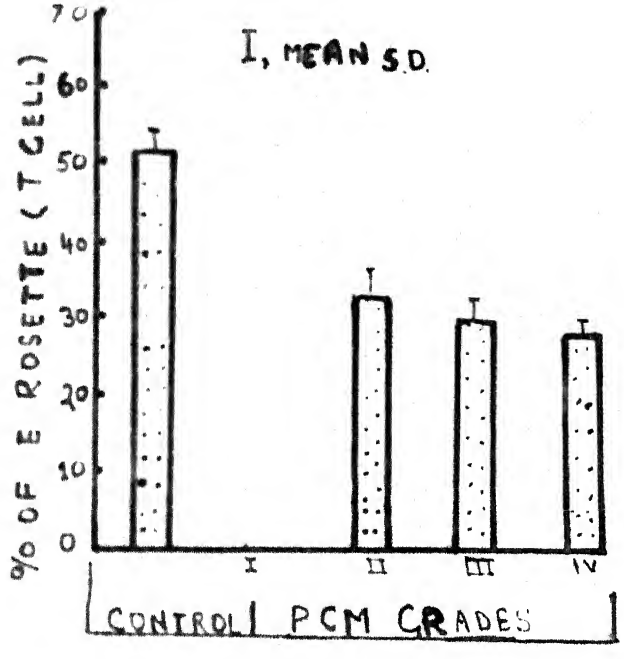


FIG 3 COMPRATIVE DIAGRAM OF E-ROSETTE IN CONTROL AND STUDY GROUP

Table XI

Distribution of T-cell (E rosette count) in study and control group.

Groups	Control group	Study group I	II	III	IV
No.of cases	9	-	3	8	10
Absolute T-cell count Mean ± S.D.	1240.02 ± 266.215	-	1105.15 ± 528.455	1350.8 ± 874.73	1122.76 ± 422.24
Statistical difference when compared to control			70.05 (P ∠0.05)	∠0.05	∠0.05
T-cell % E-rosette count Mean ± S.D.	52.0 ± 5.889	-	33.33 ± 6.944	30.5 ± 8.660	28.1 ± 4.867
Statistical difference when compared to control			70.05 (P ∠0.05)	∠0.05	∠0.05

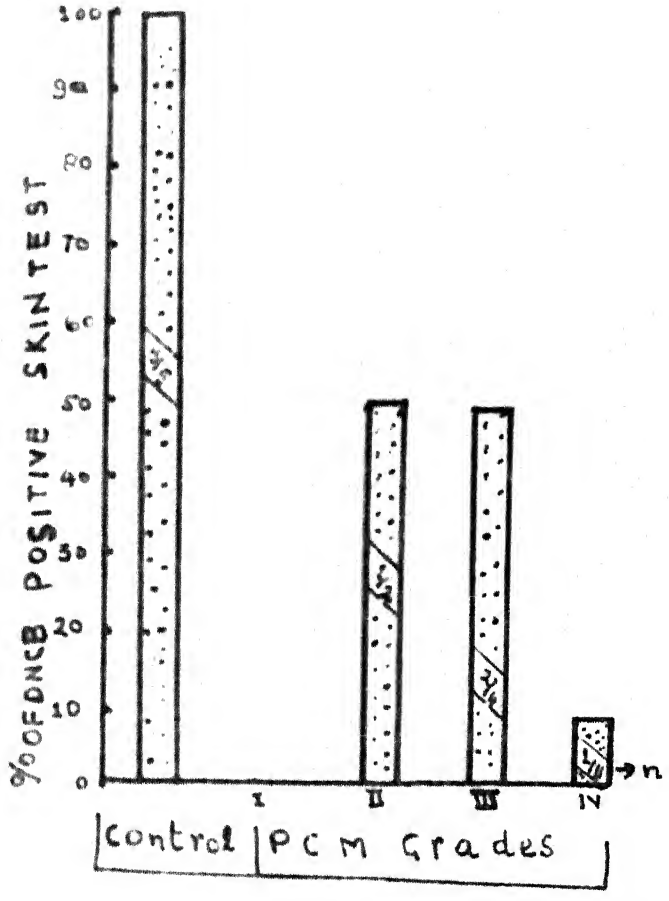
P ∠0.05 is significant

8. DNCB skin sensitizing test in control and study groups (Table XII and Fig. 4) :

To 2,4 dinitrochlorobenzene sensitization test 100% children of control group showed positive reaction while 50% of grade II and III each and only 9% of grade IV malnourished children demonstrated positive reaction.

On further examining the study group, it was observed that in grade II malnutrition, 1 out of 2 cases showed +1 positive reaction and 1 out of 2 cases showed -ve reaction to DNCB.

In grade III malnutrition, out of 4 cases 2 showed no reaction and one each case showed +1 and +2 positive reaction to DNCB. In grade IV malnutrition, 1 case showed +1 reaction and 10 cases (91%) showed no reaction to DNCB out of 11 cases in which DNCB test was done. It is evident from the table that as the degree of malnutrition increased, reaction to DNCB decreased.



4 COMPRATIVE DIAGRAM OF DNCB SKIN TEST POSITIVE CASES IN CONTROL AND STUDY GROUP.

Table XII

Delayed skin hypersensitivity test in PCM and control.

Grade of malnutrition	No.of cases	DNCB reaction +3	+2	+1	-ve	Total +ve cases (%)	Total -ve cases (%)
Control group	4	3	1	-	Nil	100	Nil
Study group :							
I	-	-	-	-	-	-	-
II	2	-	-	1	1	50	50
III	4	-	1	1	2	50	50
IV	11	-	-	1	10	9	91

Correlation between T-cell count and DNCB skin test :

It is evident from the table XIII [illegible] that, there was a gradual decrease in DNCB skin reactivity as the T-cell count decreased. T-cell count in control cases was 52.0 ± 5.889 and all the cases showed 100% positive reaction to DNCB. In II, III and grade IV malnourished cases where T cell percentages were 33.33, 30.5 and 28.1 respectively, DNCB positive reaction was observed in 50% cases each of grade II & III malnutrition and 9% of the cases of grade IV malnutrition.

Table XIII

Correlation between T-cell percentage and DNCB skin test in control and study group.

Grade of malnutrition	T-cell percentage	DNCB reaction	
		Positive (%)	Negative (%)
Control group	52.0 ± 5.889	100	Nil
Study group :			
I	-	-	-
II	33.33 ± 6.944	50	50
III	30.5 ± 8.660	50	50
IV	28.1 ± 4.867	9	91

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DISCUSSION

DISCUSSION

The present study was conducted in the department of Paediatrics, M.L.B. Medical College, Jhansi, to assess the immunological status of children suffering from protein energy malnutrition and compare the test results with those seen in healthy children.

Immunological status was assessed by B-cell count (Humoral immunity), T-cell count and DNCB skin test (Cellular immunity). Before evaluating the immunological status of children, serum albumin, total leukocyte count (TLC), differential leucocyte count (DLC), Hb%, body weight and height was assessed in every case. Data was analysed in an attempt to correlate parameters of malnutrition with the immunologic finding in children. It was also our endeavour to correlate the immunological values amongst themselves.

1. Classification of Malnutrition :

Malnourished children were identified into three clinical group on the basis of McLaren (1967) classification. Thus, the sample consisted of 13 cases of Maresmus, 5 cases of Maresmic-kwashiorkor and 3 cases of Kwashiorkor (Table I). However, cases were also

graded according to IAP (1972) Classification. Thus, 3 cases were suffering from grade II, 8 cases had grade III and 10 cases had grade IV malnutrition in the present study (Table II).

Some of the earlier workers (Hensen, 1969; Surendra Kumar, 1973 and Bhandari, 1977) had preferred to classify their malnourished cases on the basis of clinical features (score system), designating the groups as Marasmus, Kwashiorkor and Marasmic-kwashiorkor.

Rosen (1971) in his study of immunoglobulin levels in PEM used the clinical classification for assessment of nutritional status.

McMurray et al (1981) divided their cases into Kwashiorkor and Marasmus groups on the basis of clinical findings of oedema (presence or absence), serum albumin concentration and loss of subcutaneous fat.

Kumar et al (1978) in their study of cell-mediated immune response in PEM used the same criteria as that of Murray et al (1981).

Bang et al (1975) used clinical criteria for classifying under-nourished children whereby Kwashiorkor was diagnosed on the basis of oedema, puffiness of face, skin pigmentation and color/texture of hair. Similar criteria were used by Mukherjee (1967) for sub-grouping under-nourished children.

Sanjeev et al (1981) in their study of immune response (DNCB skin test) in malnourished children used the following criteria for selection of cases :

Children taking inadequate calories and protein, oedema, hair/skin changes and weight below 70% of expected weight-for-age. Certain cases excluded by the authors were those who had received immuno-suppressive therapy.

Fakhir et al (1988) in their study of serum immunoglobulins and B-cell count in PEM assessed the nutritional status of children by the same criteria as those used by Sanjeev et al and care was taken by them to exclude those cases who had associated infection.

However, certain authors (Goel et al, 1980 and Chandrasekhar et al, 1983) preferred to group their cases according to IAP (1972) classification because it was more objective.

Malnourished cases in the present study were also graded according to IAP (1972) Classification. Thus, 3 cases were suffering from Grade II, 8 cases had grade III and 10 cases had grade IV malnutrition.

Associated clinical findings, observed in malnourished children of the present study, were diarrhoea and skin lesion. On clinical examination, 15 (71.4%) cases in the present study had chronic diarrhoea, 3 (14.3%) had oedema, 3 (14.3%) had hair changes, 5 (24%) had skin changes and 8 cases (38%) had hepatomegaly (Table III).

Chandra (1983) observed in his study that the incidence of diarrhoeal illness was increased in nutritional deficiency status. Similar results were obtained by Chandra in yet another study (1985).

All the malnourished children at the time of admission were receiving diet which was grossly deficient in calories and/or protein. All the cases except control, belonged to low socio-economic status. Care was, however, taken to exclude those cases in whom secondary factors, thought to effect immune status, could have been operative.

2. Correlation of age with malnutrition :

Maximum number of malnourished children in the present study were seen in 1 - 2 and 2 - 3 year age-groups comprising (N=14) and (N=5) cases respectively, out of 21 cases examined. Similar age distribution was observed by Koster et al (1987) in their study of 225

malnourished children, where 45% children were in 1 - 2 year age and 30% children in 2 - 3 year age groups (Table V).

3. Serum albumin value in control and study groups :

Serum albumin value (4.53 ± 0.368 gm/dl) in control cases, observed in the present study (Table VIII and Fig. 1) was more or less similar to the values obtained by various other workers in the field (Mehta et al, 1972; Ronghu, 1974; Bhandari, 1977; Heller et al, 1978; Goel et al, 1980; Chandrasekhar et al, 1983; Saha et al, 1983, and Kinia et al, 1984).

It was observed in the present study that serum albumin value had a decreasing trend with the severity of malnutrition. In PCM group, mean serum albumin was 3.9 ± 1.2 gm/dl in grade II, 3.4 ± 0.2 gm/dl in grade III and 2.0 ± 0.1 gm/dl in grade IV malnutrition respectively (Table VIII).

An inverse correlation of serum albumin to severity of malnutrition has also been observed by various other workers in this field (Mehta, 1972; Surendra Kumar, 1973; Bhandari, 1977; Goel et al, 1980 and Chandrasekhar et al, 1983). However, mean serum albumin level of 3.0 ± 0.61 gm/dl in malnourished cases

reported by Olusi et al (1976), was considerably lower as compared to that observed in the present study.

Table XIV

Comparison of mean serum albumin level from different studies.

Sl. No.	Author	Year	Serum albumin (PCM) (gm/dl)			
			Control	Study group II	III	IV
1.	Neumann et al	1975	4.3 ± 0.9	3.9 ± 1.2	3.4 ± 0.2	2.0 ± 0.1
2.	Prabha et al	1978	3.55 ± 0.17	-	1.62 ± 0.43 (P < 0.001)	-
3.	McMurray et al	1981	3.96 ± 0.20	-	3.76 ± 0.18 (P < 0.05)	3.34 ± 0.26
4.	McMurray et al	1981	4.11 ± 0.58	3.63 ± 0.21	2.77 ± 0.34 (P < 0.05)	1.54 ± 0.09
5.	Present study	1989	4.53 ± 0.367	3.71 ± 0.578	3.44 ± 0.112	3.35 ± 0.534

Bell et al (1976) studied serum albumin in control and malnourished children. They observed that there was a significant reduction of serum albumin in malnourished group compared to control.

4. Haematological values in control and study groups :

Control group :

In the present study mean Hb% value was found to be 12.16 ± 0.577 gm/dl. Saha et al (1983) and Kumar et al (1984) reported in their study that mean Hb value was 12.5 ± 1.04 gm/dl and 13.1 ± 0.6 gm/dl respectively. These values were more or less similar to values observed in the present study.

PCM group :

Mean Hb values in II, III and IV grades of PCM were 11.4 ± 1.14 gm /dl, 11.0 ± 0.90 gm/dl and 10.4 ± 1.2 gm/dl respectively. There was a gradual reduction of Hb% value as the degree of malnutrition increased.

Kumar et al (1984) observed that mean level of Hb was significantly depressed in children with PCM (7.82 ± 1.62 gm/dl).

McMurray et al (1981) observed that all cases of Kwashiorkor had significantly reduced levels of Hb%.

Lymphocyte count :

The mean percentage lymphocyte count in control cases was 44.77 ± 0.941. Mean percentage lymphocyte counts in various grades of malnutrition were as follows: 40.33 ± 10.5 in grade II, 38.5 ± 14.274 in grade III and

35.3 ± 12.129 in grade IV malnutrition. These values were decidedly lower than those seen in control cases (Table IX).

A similar reduction in lymphocyte count was observed by various other workers in the field (Table XV).

Table XV

Comparison of mean lymphocyte count in normal and malnourished children by various workers.

Sl. No.	Author	Year	Lymphocyte value (%)			
			Control	II	III	IV
1.	Prabhe et al	1978	57	55	52	36
2.	Sanjeev et al	1981	46.7	-	-	43
3.	Present study	1989	44.77 ± 0.941	40.33 ± 10.5	38.5 ± 14.27	35.3 ± 12.13

Absolute lymphocyte count :

There was gradual fall in the absolute lymphocyte count as the severity of malnutrition increases, as observed in the present study. In control cases it was

3775.50 /cm, while 3485.75/cm, 3123.61/cm, and 2496.22/cm counts were observed in grade II, III and IV malnutrition respectively.

A similar reduction of absolute lymphocyte count was observed by various other workers in their studies (Table XVI).

Table XVI

Comparison of mean absolute lymphocyte counts in normal and malnourished children by various workers.

Sl. No.	Author	Year	Mean absolute lymphocyte count (per cmm)			
			Control	II	III	IV
1.	Neumann et al	1975	3270 ± 290	-	3380 ± 240	2860 ± 300
2.	Prabha et al	1978	6870 ± 750	-	-	2770 ± 300
3.	McMurray et al	1981	4596 ± 312	-	4224 ± 190	3626 ± 193
4.	Present study	1989	3775.50	3485.75	3123.61	2496.22

Results obtained in the present study are in conformity with those obtained by Vint (1937), Smyth et al (1971), Brown et al (1971) and Chandra (1972). Watts (1978) has postulated that in malnourished children atrophied thymus was the most likely cause of lymphopenia.

5. Immunological status in control and study group (PCM) :

Immunological status was studied by observing the B-cell and T-cell lymphocyte counts.

B-cell (EAC) rosette in control and study groups :

B-cell percentage and absolute counts in control and study group were estimated and have been depicted in table X and Fig. 2. The B-cell percentage and absolute counts (27.66 ± 1.805 and 686.804 ± 216.469 respectively in the control group) did not differ significantly ($P > 0.05$) from the values obtained in malnourished groups of cases. This finding is more or less similar to the finding obtained by other workers (Brown et al, 1965; McFarlane, 1970 and Prabha et al, 1978). Absolute B-cell counts were not significantly different in malnourished and healthy controls ($P > 0.05$) as observed by Cushing et al (1976), Puri et al (1980) and Fakhir et al (1983).

Keet (1969), Smythe et al (1971) and Chandra (1985) in their study observed that there was a normal B-lymphocyte percentage count in malnourished subjects as in the control cases. Also, absolute B-cell count was found to be normal in PCM and control case by the authors. Finding in the present study was also consistent with the study of above workers and some others (Smythe et al, 1971; Bang et al, 1975; Reddy et al, 1977; Chandra et al, 1977 and Vyom et al, 1980).

Table XVII

Comparison of B-cell percentage in control and study groups observed by various workers.

Sl. No.	Author	Year	Control group B-cell %	Study group B-cell %
1.	Bang et al	1975	31.30	36
2.	Prabha et al	1978	14.25	15.20
3.	Vyompuri et al	1980	27.46	25.13
4.	Fakhir et al	1988	13.36	20.51
5.	Present study	1989	27.66	27.23 -II * 25.87 -III* 27.90 -IV *

* Pooled mean : 27.04

Table XVIII

Comparison of absolute B-cell count in control and study groups as observed by various authors.

Sl. No.	Author	Year	Absolute B-cell count per c.m.m.			
			Control	PCM		
				II	III	IV
1.	Prabha et al	1978	970	-	-	-
2.	Vyompuri et al	1980	1428	1328	1204	1348
3.	Fakhir et al	1988	1479.6	1530.60	1498.76	1761.68
4.	Present study	1989	686.804 ± 216.47	812.45 ± 114.604	954.94 ± 531.09	1048.0 ± 377.64

However, Fakhir et al (1988) observed significantly raised percentage B-cell counts in malnourished children : Marasmus ($P < 0.001$), Marasmic-Kwashiorkor ($P < 0.001$) and Kwashiorkor ($P < 0.001$). But absolute B-cell counts remained unaltered ($P > 0.05$) as observed by these authors.

Prabha et al (1978) in their study observed highly reduced ($P < 0.001$) absolute B-cell counts in protein deficient animals while B-cell percentage counts remained unaltered in these animals. However, she did not comment on normal B-cell percentage counts, generally reported in PCM cases.

Smythe et al (1971), Bhuyan et al (1973) and Chandra (1974) observed in their studies that there was no significant variation in percentage B-cell count and absolute B-cell count in control and PCM cases.

McMurray et al (1981) showed in their study that B-cell (EAC) rosette percentage in PEM remained unaltered in malnourished and control groups.

T-cell (E-rosette) percentage and absolute T-cell count in control and study (PCM) groups :

T-cell percentage (E-rosette) and absolute T-cell counts observed in the present study have been depicted in table XI. It is evident from the table that both T-cell percentage and absolute T-cell count were significantly lower ($P < 0.05$) in grade III and IV of malnutrition. These were not significantly low ($P > 0.05$) in grade II malnutrition.

Prabha et al (1978) reported a significant decrease in T-cell percentage count in malnourished children (42.7 ± 2.2) as compared to control cases (47.3 ± 2.5).

Table XIX

Comparison of T-cell percentage E-rosette) in control and study groups as estimated by various workers.

Sl. No.	Author	Year	Control group	Study group
			T-cell % (mean)	T-cell % (pooled mean)
1.	Perghuson et al	1974	60	25
2.	Bang et al	1975	63	46
3.	Kumar et al	1978	66.2	33 - M 36.5 - NW
4.	Prabha et al	1978	47.8	42.7
5.	Vyompuri et al	1980	62.8	49.93
6.	Present study	1989	52.0	33.3 - II 30.63 30.5 - III 28.1 - IV

Table XX

Comparison of absolute T-cell count in control and study groups with present study from different studies.

Sl. No.	Author	Year	Control group	Study group		
				II	III	IV
1.	Kumar et al	1978	2065	792.54	855.67	-
2.	Prabha et al	1978	3025	-	1115	-
3.	Fakhir et al	1988	1479.6	1224.9	1258.2	1019
4.	Present study	1989	1240.02 ± 266.215	1105.15 ± 528.455	1350.80 ± 874.73	1122.76 ± 422.24

Bhaskaran (1974), Ferghusson et al (1974), Bang et al (1975) and Chandra (1980) observed that in the peripheral blood, number of circulating T-lymphocytes were reduced in PEM as compared to healthy control cases. Chandra (1980) further observed that this reduction was parallel with the weight loss, being 58 - 79% in control and 23% in PEM cases.

Anita et al (1968), Sellmeyer et al (1972), Seth and Chandra (1972) and Sri Sinha et al (1973) got similar results from their studies. Yet in another study, Chandra (1985) observed that there was a reduction in the percentage and absolute number of thymus dependent T-lymphocytes in cases of malnutrition.

Neumann et al (1978) also obtained result like those in the present study.

6. Cellular immune responses (DNCB) in control and study groups :

(i) Dinitrochlorobenzene skin sensitization test :

In the present study, all the subjects in the control group reacted to DNCB. While in various groups of PCM, viz., grade II, III & IV malnutrition, the percentage of positive reaction to DNCB decreased from 50% in grade II & III to 10% in grade IV. Therefore,

it was concluded that the impairment in DNCB reaction paralleled the severity of malnutrition.

Table XXI

Comparison of DNCB reaction in normal and malnourished children as reported by various workers.

Sl. No.	Author	Year	Positive reaction		Negative reaction	
			Control	PCM	Control	PCM
1.	Smythe et al	1971	19 (100)	5 (8)	-	12 (92)
2.	Chandra	1972	15 (100)	8 (34.8)	-	15 (65.2)
3.	Schlesinger and Stekel	1974	8 (88.8)	4 (33.3)	1 (11.1)	8 (66.9)
4.	Rai et al	1981	92 (86.7)	26 (54.1)	4 (13.3)	78 (45.9)
5.	Murray et al	1981	31 (100)	20 (50)	-	20 (50)
6.	Saha et al	1983	15 (100)	12 (48)	-	13 (52)
7.	Present study	1989	4 (100)	4 (23.6)	-	11 (76.4)

Figure in parenthesis indicates percentage.

The result of DNCB reaction in control and study groups as obtained by various other workers is depicted in table XXI. From the table it is evident that the observations of the present study are nearly close to those reported by Rai et al (1981), Murray et al (1981) and Saha et al (1983) who obtained 54.1%, 50% and 48% positivity to DNCB, respectively in PCM cases, while there was a 23.6% positivity to DNCB in the present study.

Also, in control cases of the present study percentage of positivity was 100% to DNCB test. This was the same as obtained in majority of other studies (Smythe et al, 1971; Chandra, 1972; Murray et al, 1981 and Saha et al, 1983).

Table XII shows that there was gradual fall of positivity to DNCB as the degree of malnutrition increased. Similarly, Sanjeev et al (1981) in their study of cell mediated immunity in malnourished cases using DNCB skin test revealed that malnourished children developed impaired reaction to DNCB. The DNCB reaction was related to degree of malnutrition.

(ii) Correlation between T-cell percentage and DNCB reactivity (Table XIII & Fig. 2):

The present study revealed that T-cell percentage was positively correlated to DNCB reaction. Control cases,

where T-cell percentage count was 52.0 ± 5.889, showed 100% positivity to DNCB. In grade II, III and IV malnutrition T-cell percentage counts was 33.33 ± 6.944, 30.5 ± 8.660 and 28.1 ± 4.867 respectively. DNCB reactivity was 50% in grades II and III malnutrition, while in grade IV malnutrition only 9% cases showed positive reaction and the rest showed negative reaction. Finding of the present study are in conformity with those reported by Rai et al (1981), Murray et al (1981) and Saha et al (1983).

Various hypotheses have been put forward by different workers from time to time giving explanations for the low reactivity to DNCB, low value of T-cell percentage (E-rosette) and non-significant change in B-cell percentage in cases of PEM.

Dietary factor :

Most of the authors (Smythe et al, 1964; Hansen et al, 1964; Goel et al, 1980) are of the opinion that diet deficient in protein and calories was one of the prime factors in causating low immune response.

Decreased intestinal absorption :

The factor of decreased intestinal absorption has been highlighted by nearly all the workers in the

field (Tomkin et al, 1981; Robin et al, 1976; Bang et al, 1975; Mukherjee, 1967; Koster et al, 1987; Kumar et al, 1978) as being another important factor for low level of T-cell % in PEM. This fact has also been demonstrated by observations in the present study; that cases of PEM had more diarrhoea.

Skin test reactivity, however, may simply correlate with non-specific intestinal integrity such as epithelial turnover or with the ability to amplify more specific immune factor production in order to focus a mucosal inflammatory response. Altered skin test reactivity may reflect impaired lymphocyte mediated diffuse response in the gut mucosal wall, about which little is known (Brunser et al, 1968).

The differential depression of cell mediated immunity is consistant with lymphoid tissue atrophy which may be the main site of T-cell output. The B-cell production probably is a function of bone marrow which is often active in these under-nourished patients, thus explaining a normal humoral immune mechanisms. This explanation was also supported by Vyompuri et al (1980).

Lymphocyte response to a mitogen such as PHA, DNCB depends on the quantity of T-cells in the culture. It seems that low number of rosette forming cells explains the impairement of DNA synthesis and also the

failure to develop delayed reaction to DNCB. This has also been seen in states of primary immune deficiency in which the thymus is absent or functionally deficient (Wybran & Fundenberg, 1973; Chandra, 1974).

Depression of CMI in PCM could be the result of an absolute or relative deficiency of an amino-acid necessary for cell multiplication (Smythe et al, 1970). Watts (1969) has postulated that in malnourished children, the atrophied thymus is most likely the cause for impaired CMI response.

Axelord (1971) has reported decreased cellular immunity in animals rendered iron deficient. Anaemia produced in adults has also been reported to decrease CMI (Joynson, 1972).

It seems poor intake, diarrhoea-induced malnutrition and damaged intestinal mucosa secondary to severe PCM; all contribute to decrease in CMI.

Ferghuson et al (1974) noted in children with PCM, a profound but diet reversible depression of the ability of thymus derived lymphocyte to form rosettes with sheep erythrocytes, before lymphopenia or abnormalities of in-vitro tests could be detected. As protein calorie malnutrition became more, interference with cell growth and cell division occurred with the

result that lymphopenia, decreased tonsillar size and impairment of in-vitro lymphocyte proliferation occurred.

McMurray et al (1981) opined that a significantly reduced number of lymphocytes in grade II malnutrition in children might reduce the degree of delayed hypersensitivity. Atrophy of lymphoid tissue has long been recognized as a hall mark of severe malnutrition and also an explanation for impaired immune function (Benitz-Bribiesca et al, 1975). Protein malnutrition in early developmental period, it seems, has a marked influence on lymphoid development. Data in the present study suggests that reduced number of peripheral T-lymphocytes could be responsible for reduced skin DNCB reactivity and hypertrophy of secondary lymphoid tissues, like the tonsil. Probably, T-cells per-se are capable of responding but due to their reduced population in individuals, subjected to PCM, the full extent of the cellular immune response is not expressed. This view has been supported by Prabha et al (1978).

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SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The present study was conducted at M.L.B. Medical College, Jhansi, over a period of one year to assess the immune status of malnourished children.

Twenty one malnourished children of 1 - 5 years of age were selected and the results were compared with 9 healthy control subjects of the same age group. Immuno-competence in these subjects was assessed by estimating T-cell percentage, B-cell percentage and dinitrochlorobenzene skin sensitization test (DNCB). A detailed clinical examination, physical anthropometry biochemical and pathological investigations were done to estimate haemoglobin, total leucocyte count, differential leucocyte count, serum albumin, urine albumin and sugar in these subjects. Those cases were excluded from the study who had received immuno-suppressive drugs or else were suffering from a disease which effected immunity.

Malnourished children when sub-grouped according to Wellcome Classification (1967) were categorized into marasmus (N = 13), marasmic kwashiorkor (N = 5) and kwashiorkor (N = 3). All PCM children had body weight less than 70% of the NCHS standard for age. PCM cases

were also categorized according to IAP (1972) Classification of malnutrition. Thus, there were 3 cases who belonged to grade II, 8 cases to grade III and 10 cases to grade IV malnutrition.

The results obtained, following statistical analysis of the data, are summarized below :-

T-lymphocyte and B-lymphocyte counts

1. Control group

The mean T-lymphocyte and B-lymphocyte percentage counts in control cases were 52 $\pm$ 5.888 and 27.66 $\pm$ 1.805 respectively.

2. PCM group

Mean T-lymphocyte percentage count was 33.33 $\pm$ 6.94 in grade II, 30.5 $\pm$ 8.660 in grade III and 28.1 $\pm$ 4.867 in grade IV malnutrition. All these values were significantly lower in comparison to the value observed in control cases. The difference was statistically significant ($P < 0.05$) in grade III and grade IV cases of malnutrition, while it was not significant in grade II cases of malnutrition. The level of T-lymphocyte percentage was inversely proportional to the degree of malnutrition.

Mean B-lymphocyte percentage (EAC rosette) values in different grades of malnutrition were 27.33, 25.87 and 27.90 in grade II, III & IV cases respectively.

There was no significant difference ($P > 0.05$) in B-lymphocyte percentage counts, between the control and PCM groups of cases.

DNCB skin test

DNCB skin test was done to assess the cellular immunity.

1. Control group

All the cases in control group showed positive reaction to DNCB skin test. Thus, the cellular immunity was considered as being intact in these healthy cases.

2. PCM group

Half the cases (50%) in grade II & III malnutrition showed positive skin test to DNCB, while only 9% cases of grade IV malnutrition had positive skin reaction.

Positive reaction to DNCB was inversely proportional to severity of malnutrition.

Correlation of T-cell percentage and DNCB reaction

1. Control group

Mean T-cell percentage was 52.0 ± 5.89 and all the cases in this group showed positive reaction to DNCB.

2. PCM group

T-cell percentage was 33.33 ± 6.94, 30.5 ± 8.660 and 28.1 ± 4.87 in II, III and IV grades of malnutrition respectively. Out of 2 cases of grade II malnutrition studied, one case showed +1 reaction and one case showed -ve reaction to DNCB skin test. Out of four cases of grade III malnutrition, one case showed +2 and another one showed +1 reaction, while 2 cases showed negative reaction. Out of 11 cases of grade IV malnutrition, 10 cases showed negative reaction and only one case showed +1 reaction to DNCB skin test. Thus we concluded that DNCB skin test was directly correlated with T-cell percentage.

Serum albumin, serum haemoglobin, total lymphocyte count and differential cell count

1. Control group

Mean serum albumin - 4.53 ± 0.367 gm/dl.

Mean haemoglobin - 12.16 ± 0.577 gm/dl.

Mean lymphocyte percentage - 44.77 ± 0.941

Absolute lymphocyte count - 3775.50 /mm^3

2. PCM group

	Grade II	III	IV
Mean serum albumin (gm/dl)	3.71	3.44	3.35
Mean haemoglobin (gm%)	11.40	11.0	10.4
Absolute lymphocyte count	3485.75	3123.60	2496.22
Percentage lymphocyte count	40.33	38.5	35.3

There was a gradual decrease of haemoglobin, serum albumin, absolute lymphocyte count and percentage lymphocyte count as the severity of malnutrition increased.

CONCLUSION

1. In healthy children of 1 - 5 years age group, no significant variation in immunological parameters viz., T-cell, B-cell and DNCB reactivity occurred owing to age differential.

2. PCM cases had significantly decreased level of haemoglobin, absolute and differential lymphocyte counts/serum albumin as well as low T-lymphocyte counts, when results were compared to normal healthy cases of the same age group.

3. There was no significant difference in B-lymphocyte count between control and PCM groups.

4. DNCB reactivity showed direct relation to T-lymphocyte count. DNCB skin test positivity was inversely proportional to the degree of malnutrition.

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A P P E N D I X

APPENDIX

STUDY OF CELL MEDIATED IMMUNITY IN PROTEIN ENERGY MALNUTRITION

CASE PROFORMA

Case No. M.R.D.No. :

Name : Age/Sex: D.O.B.

Father's Name :

Address :

Occupation : Father : Mother:

Total income of family: Rs. /Month

Per capita income Rs. /Month

Present history :

Family history :

Past illness :

(1) Fever
(2) Chronic diarrhoea
(3) Failure to thrive
(4) Worm infestation
(5) Cough
(6) Vomiting
(7) Pertussis
(8) Measles
(9) BCG Vaccination Yes/No
(10) History of taking steroid

Geniological tree :

Dietary History :

Diet	Age at starting	Upto age	Dilution
Breast milk			
Artificial milk			
Added artificial milk			
Solid added			

Present diet :

Calories Protein Adequate/Inadequate

Immunization history :

	Smallpox	BCG	Polio	DPT
I				
II				
III				

Antenatal, Natal and Post-natal history :

DEVELOPMENTAL HISTORY :

MOTOR Age :

(1) Head control

(2) Sitting

(3) Crawling

(4) Standing With support
Without support

(5) Walking

(6) Running

Manipulative

(1) Grasp

(2) Self feeding : Spoon
Cup

(3) Help in dressing

Social

(1) Smile (Social)

(2) Response to call by name

(3) Sphinter control

Bladder	day	night
Bowel	day	night

Speech

(1) Single word (mama, etc.)

(2) Jargon

(3) Small broken sentences

(4) Long sentences

CLINICAL EXAMINATION :

General appearance		Healthy / Malnourished
Psychomotor change		Irritable / Apathetic
	Hair :	Texture
		Colour
		Easy pluckability
		Sparseness
		Brittile
Face		Moon face
Eye		Conjunctival xerosis
		Bitot's spot
		Pale conjunctiva
Mouth		Angular stomatitis
		Chelosis
		Glossitis
		Bleeding gums

Dentition

Skin
- Oedema
- Follicular hyper-keratosis
- Pellagrous dermatosis
- Flaky paint dermatosis
- Diffuse dipigmentation
- Mosaic dermatosis

Subcutaneous fat :

Muscle fat :

Skeletal system :
- Epiphyseal enlargement
- Rickety rosary
- Anterior fontandle
- Harrison's sulcus
- Frontal bossing
- Knock knee
- Bow leg

Abdomen :
- Liver
- Spleen
- Pot belly
- Others

Cardio-vascular system :

Respiratory system :

Central Nervous system :

Anthropometric measurement :

Weight kg.
Crown heel
Length/Height cm.

Investigation :

Blood :

TLC - /cu.mm.
DLC -
Hb - gm %